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School of Engineering

Institute of Infrastructure and Environment

The impact of silver and titanium dioxide nanoparticles on the in-vessel composting of biodegradable municipal solid waste

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DECLARATION

I declare that this thesis and the work presented in it are my own and that it has been generated by me as the result of my own original research under the supervision of Dr Blanca Antizar-Ladislao. I confirm that: (i) this work was done while in candidature for a research degree at the University of Edinburgh; (ii) where I have consulted the published work of others, this is always clearly attributed; (iii) where I have quoted from the work of others, the source is always given; with the exception of such quotations, this thesis is entirely my own work; (iv) I have acknowledged all main sources of help; and (v) where the thesis is based on work done by myself jointly with others I have made clear exactly what was done by others and what I have contributed myself.

Signed: ……

Date: ……

To my lovely wife Konstantina and our little baby Sissy

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Η διδακτορική ερεύνα χρηματοδοτήθηκε απο το
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ABSTRACT

The extensive use of nanoparticles (NPs) has started receiving increased attention because of the knowledge gaps regarding their fate in the environment and the possible impact on the environment and human health. The production of silver nanoparticles (AgNPs) and titanium dioxide nanoparticles (TiO_2-NPs) is increasing and it is expected that, due to their great number of applications, their concentration in waste streams will increase in the future. The presence of NPs in waste streams may affect the treatment process (e.g., composting) and, if they are not successfully removed from the waste streams, their presence in the treated waste (e.g., compost) may present an environmental risk. Composting of the biodegradable fractions of municipal solid waste (MSW) is a widely used waste management practice, mainly because it is a cost-effective treatment technology and the final product (i.e., compost) presents several benefits to the environment, particularly as a soil conditioner.

The overall aim of this thesis is to assess the effect of Ag-TiO_2NPs and AgNPs that may be present in the biodegradable fractions of municipal solid waste on composting and subsequent soil application of compost. For that purpose in-vessel composting of artificial municipal solid waste contaminated with commercial nanoparticles was investigated at laboratory scale, simulating a range of relevant concentration levels. Subsequently, the fate of NPs present in mature compost use as a top-layer soil conditioner was investigated using a column approach at laboratory scale. The toxicity effect of NPs present mature compost on plant growth was further investigated. The impact of NPs during composting was assessed by monitoring the temporal dynamics of organic matter (OM) using Excitation Emission Matrix (EEM) fluorescence spectroscopy. The fate of NPs following application of contaminated mature compost as a top-soil conditioner and potential release to groundwater was investigated using a column leaching experiment while the phytotoxicity of mature compost contaminated with NPs was assessed using a seed germination bioassay. Finally, to investigate further possible environmental impacts due to the application of mature compost contaminated with NPs to soils, a Life Cycle Assessment (LCA) was conducted.

The impact of commercial Ag-TiO_2 NPs and AgNPs on the in-vessel composting of biodegradable municipal solid waste was investigated over 21 days, using initial concentrations of 0, 5, 10, 20 and 50 mg Ag / kg of OM. Microbial activity was

inhibited in the biodegradable waste reactors using 2% NaN_3 to evaluate abiotic losses. Physicochemical parameters such as pH, ash content, weight loss, and the formation of humic substances (HS) were determined after 0, 4, 7, 14 and 21 days of composting and after a maturation phase. The results indicated that the presence of 2% NaN_3 in biodegradable MSW inhibited effectively the microbial activity during the first week of composting. The microbial population was activated during the second week of composting but the decomposition rate was so low that did not result in the formation of humic substances (HS) following 21 days of composting when 2% NaN_3 was used. Both treatments, using Ag-TiO_2-NPs and AgNPs, did not show any inhibition of the decomposition process for all the tested concentrations and EEM peaks shifted towards the HS region during in-vessel composting. Higher inorganic carbon removal resulted from NP-contaminated compost with higher NP concentrations. This may indicate that the formation of humins was higher for non-contaminated compost and decreased as the NP concentration in waste increased. The shift of the peaks towards the HS region during composting for all the treatments suggested that NPs did not have an effect on humification and therefore on compost stability.

The leaching properties of the NP-contaminated compost were investigated using a column leaching test. Five samples of leachate, of 50 mL each, were collected. The highest concentrations of HS were observed in the first two leaching samples. The leaching results suggested that only a low percentage of the total NPs (in weight) in compost, up to *ca.* 5% for Ag and up to *ca.* 15% for Ti, leached out from the columns, which was assumed the amount that potentially could leach to the environment. These results suggested that NPs will mainly accumulate in soils' top layers following application of compost contaminated with NP. The phytotoxicity of NP-contaminated compost was assessed using a seed germination bioassay and the germination index was then calculated. The results indicated that the NP-contaminated compost did not present any toxic effects to cress germination.

The possible environmental impacts due to the NP-contaminated compost application to soils were investigated by conducting a comparative LCA study. The LCA study indicated that the effects of NP-contaminated compost to human health and ecosystems endpoint categories increased due to the presence of NPs. The risks are associated with terrestrial ecotoxicity and human toxicity midpoint categories and are mainly attributed to the accumulation of Ag to soils.

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NOTATION

AgNPs : Silver nanoparticles

Ag-TiO_2NPs : Silver doped titanium dioxide nanoparticles.

UPW: Ultra Pure Water

DLS: Dynamic Light Scattering

DOM: Dissolved Organic matter

EEM: Excitation Emission Matrix

E: Egalitarian

EU-28: European Union of 28 member states

FA: Fulvic Acid

FET: Freshwater Eco-toxicity

HA: Humic Acid

H: Hierarchist

HH: Human Health

HS: Humic Substances

HT: Human Toxicity

HU: Humins

ICP-MS: Inductively Coupled Plasma-Mass Spectroscopy

ISO: International Organization for Standardization

LCA: Life Cycle Assessment

LCIA: Life Cycle Impact Assessment

LCI: Life Cycle Inventory

LCSA: Life Cycle Sustainability Analysis

LCT: Life Cycle Thinking

MET: Marine Eco-toxicity

MSW: Municipal Solid Waste

NMs: Nanomaterials

NOM: Natural Organic Matter

NPs : Nanoparticles.

OM: Organic Matter

TET: Terrestrial Eco-toxicity

TiO_2NPs: Titanium dioxide nanoparticles

CHAPTER 1: SCOPE OF THE RESEARCH

1.1 Introduction

1.1.1 Solid waste management

The management of municipal solid waste (MSW) and the environmental impacts it causes are subjects gaining increasing attention in industrialised countries. The United Nations General Assembly has pointed out that waste management is a major factor in maintaining the quality of the Earth's environment and achieving sustainable development (UN 1992).

Waste generation has been growing steadily worldwide over the last few decades due to population growth, urbanisation and industrialisation. The current world population of close to 7 billion is projected to reach 10.1 billion in the next ninety years, reaching 9.3 billion by the middle of this century (UN 2011). According to the US Environmental Protection Agency, the generation of MSW in the US has increased from *ca.* 152 million tons in 1980 to *ca.* 243 million tons in 2009 (USEPA 2010). MSW generation in EU (15 countries) has increased from *ca.* 203 million tons in 1998 to *ca.* 217 million tons in 2009 (Eurostat 2011). In England it was estimated that MSW generation in 2006/2007 was *ca.* 28 million tons (Defra 2009).

Current MSW management practices include recycling, sanitary landfill, thermal treatment (incineration with the recovery of energy, gasification and pyrolysis) and biological treatment (composting and anaerobic digestion). Landfill was the main waste disposal method in the past and it continues to be for many countries around the world. From open dumps to sanitary and controlled landfill sites, this has resulted in an ever surmounting accumulation of waste in the environment, parts of which will take centuries to biodegrade. EU Directive 1999/31/EC on the landfill of waste sets targets to reduce the amount of biodegradable municipal waste sent to landfill to 75% of 1995 figures by 2010 and to 35% by 2020 (EU 1999). By diverting organic waste from landfill sites, the production of landfill gas (i.e., CH_4, CO_2) is minimised (Chapman and Antizar-Ladislao 2009). High technology composting, including waste separation, is an alternative, more cost effective, waste management practice compared to landfill for the biodegradable fraction of MSW. The application of compost as a soil conditioner or fertilizer can reduce greenhouse gas emissions by

sequestering biogenic carbon in soils. This improves the physical properties of the soil and restores essential nutrients back to the soil (ISWA 2010).

Sustainable Waste Management SWM is therefore gaining increased attention in order to both reduce the environmental impact caused by the uncontrolled biodegradation of waste materials, as well as the challenge of resource reuse and recovery.

The aim of the EU Sustainable Development Strategy (SDS), which was adopted by the European Council in 2001 and last reviewed in 2009 (EU 2009), is to support and promote actions to achieve the continuous improvement of quality of life for both current and future generations. This strategy consists of a framework with a long term vision of a sustainable system which brings new challenges to Solid Waste Management Systems (SWM). This is being introduced through the implementation of the EU Directive 2008/98/EC on waste (EU 2008).

Life Cycle Thinking (LCT) is a core concept for Sustainable Consumption and Production for both businesses and policy development. The environmental pillar of LCT is supported by Life Cycle Assessment (LCA), an internationally standardised tool for the integrated environmental assessment of products (goods and services) (ISO 2006a, ISO 2006b). According to ISO 14040 (ISO 2006a), "Life cycle assessment is the compilation and evaluation of the inputs, outputs and the environmental impacts of a product (any goods or services) system throughout its life cycle". LCA has been characterised as a unique assessment tool to reconcile development with environmental protection (UN 1992). LCA is used successfully to identify and quantify the environmental impacts of the various waste management technologies.

1.1.2 Emerging contaminants

The evolution in patterns of consumption and production has introduced emerging contaminants into waste streams that may engender sanitary and environmental risks as they may act differently from well-known and regulated substances (Marcoux, Matias et al. 2013). Emerging contaminants or contaminants of emerging concern include flame retardants, pesticides, pharmaceuticals, plasticisers, various fluorinated compounds and nanomaterials (Sauve and Desrosiers 2014). Nanomaterials (NMs) are classified as emerging contaminants due to the lack of environmental health and safety data available on them. There is currently no evidence that any nano-enabled

products cause harm. In the case of nanotechnologies the position is complicated by the potential number of NMs in circulation; where each NM may have different properties, uses and pathways to exposure. The increased use of NMs will however introduce nanoparticles (NPs) either intentionally and/or unintentionally into waste streams. Some of the waste streams generated by nanotechnology manufacturers are and will consist of purely conventional waste materials, but others will include NMs. These may then enter a landfill site, incinerator or other waste disposal technology or end-of-life scenario (Woodrow 2007). At present no nanowaste is regulated by authorities as hazardous. The Woodrow Wilson International Center's Project on Emerging Nanotechnologies (PEN) issued a report to highlight the end-of-life regulation of nanotechnologies. It's main aim being to examine how nanowaste could be controlled under existing laws in the United States (Wilson 2011).

1.2 Research Hypothesis, Aim, Objectives and Methodology

1.2.1 Hypothesis, overall aims and objectives of the research

The extensive use of NMs has started receiving increased attention because of the knowledge gaps regarding their fate in the environment and the possible impact on human health. Current waste treatment practices are not designed to consider the presence of emerging contaminants, such as NMs, in the waste. The presence of emerging contaminants in waste streams may cause environmental risks (e.g. release into the environment) or may have an impact on the treatment process (e.g. inhibition). Composting is a widely used waste management practice because it is cost-effective and has several benefits to the environment. Silver nanoparticles (AgNPs) have antibacterial properties and as a result it is expected that they will have an impact on the process when entering in through organic waste streams. As the production of AgNP and titanium dioxide nanoparticle (TiO_2-NP) is increasing, it is expected that their concentration in these waste streams will increase in the future. These assumptions are based on known issues pertaining to the accumulation of heavy metals in soils and their subsequent leaching into ground or surface waters as a result of high levels of these compounds being present in when applied to soils as a fertiliser.

The hypothesis of this thesis can be stated as:

"As AgNPs have antibacterial properties, it is therefore expected that they will have an impact on the composting process. Their subsequent release into the environment through the application of compost to the soil could either induce toxicity to plants, accumulate in the soils and/or leach into groundwater systems"

This thesis aims to assess the impact of silver doped titanium dioxide nanoparticles (Ag-TiO_2NPs) and silver nanoparticles (AgNPs) on the in-vessel composting of biodegradable MSW, the NPs fate and toxicity in the environment and the positive and negative environmental impacts of compost application to soils due to the presence of AgNPs and Ag-TiO_2NPs in waste streams.
The objectives of this thesis are as follows:

(a) Review current state of knowledge,
(b) Laboratory scale experiments to simulate an in-vessel composting process to investigate the effect of Ag-TiO_2NPs, at different concentrations, in the composting process,
(c) Laboratory scale experiments to simulate an in-vessel composting process to investigate the effect of AgNPs, at different concentrations, in the composting process,
(d) Leaching experiments, using NP contaminated compost, to assess the leaching properties of the contaminated compost and the fate of NPs in the environment,
(e) Germination tests to assess the phytotoxicity of NP contaminated compost,
(f) Data analysis using statistical tools and software,
(g) Development of a comparative LCA to investigate the positive and negative environmental impacts of compost due to the presence of AgNPs and Ag-TiO_2NPs in waste streams.
(h) Dissemination of results: publications, thesis, report.

1.2.2 Methodology of the Thesis

The methodology of this thesis was constructed in order to assess the potential impact of NPs on the composting process, and the effect of NP contaminated compost on the environment and human health.

The main aim of the experimental research was to assess the impact of NPs on the in-vessel composting of biodegradable MSW. Artificial waste, of known composition, was used for the experiments to obtain reliable and comparable results. Two sets of experiments were conducted using commercial Ag-TiO_2NPs and AgNPs. Microbial activity was NaN_3 inhibited to assess the abiotic losses. The monitoring of organic matter temporal evolution using Excitation Emission Matrix Fluorescence Spectroscopy was the main method applied to assess the impact of NPs on the composting process.

The potential effect of NP-contaminated compost used in the environment and its impact on human health and ecosystems was assessed through experimental research and work on the desk. The experimental research consisted of leaching tests to investigate the leaching potential and properties of NP contaminated compost. Seed germination bioassays were used to investigate the NPs phytotoxicity. Available phosphorus, ammonia, nitrate and nitrite of mature compost was detected to assess possible effects on surface or ground water. Desk work included the analysis of the experimental results using a LCA approach. The experimental results were implemented in the Life Cycle Inventory (LCI) to produce a comparative LCA study of composting NP-contaminated biodegradable MSW and subsequent application of NP-contaminated compost to soils.

1.2.3 Layout of the Thesis

This thesis consists of six main chapters: the scope of the research, literature review, experimental work, LCA analysis, discussion and conclusions.

The scope of the research is stated in Chapter 1. An introduction to: solid waste management sustainability, emerging contaminants in waste and LCA's feature in the scope of the research. The main aim, objectives and hypothesis of the research are also thoroughly described in this section.

A literature review of the current state of the research is presented in Chapter 2. The current MSW generation and management in EU is given, focusing on the biodegradable fraction of MSW. An introduction to composting describing the process, the parameters, the different stages and the formation of HS is presented. The emerging contaminants in waste introduces the terms of NMs and NPs, their synthesis, properties and applications, their fate in environment and their potential

toxicity. Fluorescence spectroscopy as a method used to assess the compost's stability is presented at the end of Chapter 2.

The experimental research is presented in Chapter 3 and split into two main parts. The experimental results obtained from the in-vessel composting of NP-contaminated biodegradable MSW are presented in the first part. The experimental work on NP leaching and its impact on seed germination are presented in the second part. The experimental results are thoroughly discussed and conclusions made at the end of the chapter.

A comparative LCA study is presented in Chapter 4. The experimental results obtained in Chapter 3 are implemented in the LCI to produce a comparative LCA study to assess the environmental impacts of composting NP-contaminated biodegradable MSW and application of produced NP-contaminated compost to soils.

The overall results of the thesis are discussed in Chapter 5. Conclusions and future research recommendations are made in Chapter 6.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

National and international targets on MSW focus to divert waste from landfill, to reduce waste generation and to increase recycling and recovery rates. Composting of the biodegradable fraction of MSW is a cost effective and environmentally sound treatment method. Both composting and anaerobic digestion are the main waste treatment practices meeting the goal of diverting biodegradable MSW from landfills. The presence of NMs in these municipal waste streams may have an impact on the composting process. Some of the NMs contain heavy metals thus their presence in waste can cause a degree of inhibition or interference with the process. The impact of emerging contaminants in the composting process can be assessed by applying tools used to determine the stability of the compost, such as monitoring the formation of humic substances (HS) using fluorescence spectroscopy. Furthermore, the application of compost to soils may cause environmental risks such as heavy metal accumulation in soils or the leaching of these heavy metals into ground or surface waters.

2.2 Biodegradable Municipal Solid Waste Management in Europe

The term "waste" is defined as any substance or object which the holder discards, intends to or is required to discard (EU 2008). The United States Environmental Protection Agency (USEPA) defines municipal waste as the materials traditionally managed by the municipalities, whether by burning, burying, recycling or composting (USEPA 2010). The biodegradable fraction of MSW is called bio-waste and includes the biodegradable garden and park waste, the food and kitchen waste from households, retailers (restaurant, caterers, etc.) and food processing plants (EU 2008). The biodegradable MSW (bio-waste) is differentiated from the biodegradable waste and is defined, according to the EU landfill directive, as "any waste that is capable of undergoing anaerobic or aerobic decomposition, such as food and garden waste, and paper and paperboard" (EU 1999).

Waste management is defined as the collection, transport, recovery and disposal of waste, including the supervision of such operations and the aftercare of disposal sites (EU 2008). MSW management is usually a public issue organised by the governing authorities, although some functions of the system are operated by the private sector

such as collection, plants operation, transport etc. Waste management plans set out the current waste management in a geographical entity and the appropriate measures to improve reuse, recycle, recover and dispose of waste. Current policy on MSW management, at national and international level, set targets to divert wastes from landfill and to increase recycling and recovery rates.

2.2.1 Biodegradable Municipal Solid Waste Classification

The European waste catalogue (EWC) classifies and categorises waste materials (EU 2000a). MSW classification according to the EWC is given in Table 2-1. A fraction of MSW (paints, solvent-based cleaners, oils, batteries, pesticides etc.) is characterised as hazardous and marked with an asterisk in the EWC. These wastes are not accepted in landfills according to the EU directive on landfill even if diluted or mixed to meet the waste criteria determined in accordance with Annex II of the directive (EU 1999).

2.2.2 Biodegradable Municipal Solid Waste Generation, Composition and Characteristics in European Union of 28 members states (EU-28).

According to the Organisation for Economic Co-operation and Development (OECD) the generation of MSW is increasing every year as the population and the generation per capita increases. MSW quantities generated in EU-28 for 2011 are given in Fig 2-1. MSW generation per capita in EU-28 for 2011 was 1.36 Kg/capita (Eurostat 2011). The volumes of MSW generated per capita depends on a number of factors such as the lifestyles of the population, their relative standards of living, general consumer patterns, and the level of technological advancement of a particular country. Three main factors affect the MSW generation per capita: socioeconomic status, degree of urbanisation and household size. Rural areas often have lower per-capita generation rates than urban areas for at least some components in the waste stream (for example the generation of packaging waste is lower in rural as opposed to urban areas, where packaging is very popular).

Table 2-1. Municipal waste categorized and classified according to the European Waste Catalogue (EU 2000a).

20	**Municipal Wastes (Household waste and similar commercial, industrial and institutional wastes) including separately collected fractions**
20 01	**Separately collected fractions (except 15 01)**
20 01 01	Paper and card board
20 01 02	Glass
20 01 08	Biodegradable kitchen and canteen waste
20 01 10	Clothes
20 01 11	Textiles
20 01 13*	Solvents
20 01 14*	Acids
20 01 15*	Alkalines
20 01 17*	Photochemicals
20 01 19*	Pesticides
20 01 21*	Fluorescent tubes and other mercury-containing waste
20 01 23*	Discarded equipment containing chlorofluorocarbons
20 01 25	Edible oil and fat
20 01 26*	Oil and fat other than those mentioned in 20 01 25
20 01 27*	Paint, inks, adhesives and resins containing dangerous substances
20 01 28	Paint, inks, adhesives and resins other than those mentioned in 20 01 27
20 01 29*	Detergents containing dangerous substances
20 01 30	Detergents other than those mentioned in 20 01 29
20 01 31*	Cytotoxic and cytostatic medicines
20 01 32	Medicines other than those mentioned in 20 01 31
20 01 33*	Batteries and accumulators included in 16 06 01, 16 06 02 or 16 06 03 and unsorted batteries and accumulators containing these batteries
20 01 34	Batteries and accumulators other than those mentioned in 20 01 33
20 01 35*	Discarded electrical and electronic equipment other than those mentioned in 20 01 21 and 20 01 23 containing hazardous components (6)
20 01 36	Discarded electric and electronic equipment other than those mentioned in 20 01 21, 20 01 23 and 20 01 35
20 01 37*	Wood containing dangerous substances
20 01 38	Wood other than that mentioned in 20 01 37
20 01 39	Plastics
20 01 40	Metals
20 01 41	Wastes from chimney sweeping
20 01 99	Other fractions not otherwise specified
20 02	**Garden and park wastes (including cemetery waste)**
20 02 01	Biodegradable waste
20 02 02	Soil and stones
20 02 03	Other non-biodegradable wastes
20 03	**Other municipal wastes**
20 03 01	Mixed municipal waste
20 03 02	Waste from markets
20 03 03	Street cleansing residues
20 03 04	Septic tank sludge
20 03 06	Waste from sewage cleaning
20 03 07	Bulky waste
20 03 99	Municipal wastes not otherwise specified
Any waste marked with an asterisk is considered as a hazardous waste.	

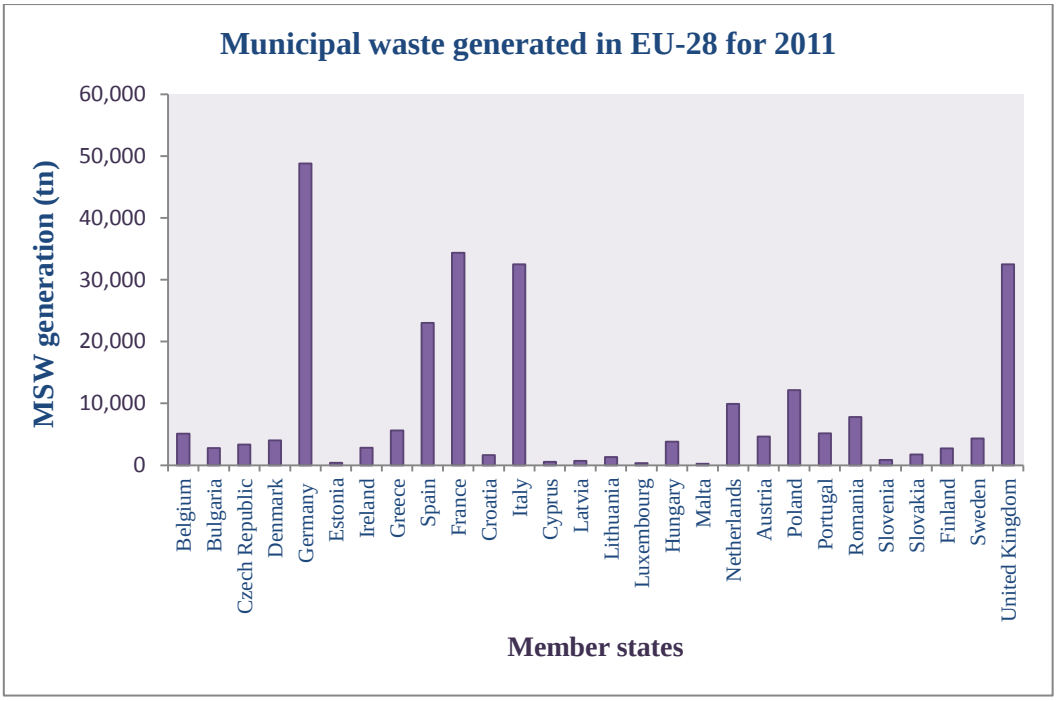

Fig 2-1. MSW generated in EU-28 for the year 2011 (Eurostat 2011).

The composition of MSW depends on similar factors such as waste generation. Waste compositional information is required to plan, implement and monitor waste management schemes at a local and national level. Waste classification's which are used to collect data may vary widely in different regions and countries. Regional data on MSW composition based on a wet weight basis, excluding yard waste and nappies, are given in Table 2-2. There is a clear difference observed between food waste and packaging waste generated between the global regions including less developed and more developed regions of the world.

Table 2-2. MSW composition data by percent in different regions (IPCC 2006).

Waste/Region	Europe		Asia		Africa		America	
	North	South	East	West	North	South	North	South
Food waste	23.8	36.9	26.2	41.1	51.1	23.0	33.9	44.9
Paper/Cardboard	30.6	17.0	18.8	18.0	16.5	25.0	23.2	17.1
Wood	10.0	10.6	3.5	9.8	2.2	15.0	6.2	4.7
Textile	2.0	n/a	3.5	2.9	2.5	n/a	3.9	2.6
Rubber/leather	n/a	n/a	1.0	0.6	n/a	n/a	1.4	0.7
Plastic	13.0	n/a	14.3	6.3	4.5	n/a	8.5	10.8
Metal	7.0	n/a	2.7	1.3	3.5	n/a	4.6	2.9
Glass	8.0	n/a	3.1	2.2	2.0	n/a	6.5	3.3
Other	n/a	n/a	7.4	5.4	1.5	n/a	9.8	13.0
n/a: not available								

More detailed data on MSW composition and biodegradable MSW content for England during 2006-2007 are given in Table 2-3. Food, garden, paper and plastic waste constitute the main fraction of MSW. Following separation, organic waste streams usually undergo biological treatment (composting and anaerobic digestion), paper and plastic are processed to refuse derived fuel (RDF) and metals are widely recycled to metalworking plants.

Table 2-3: Composition of MSW and biodegradable content in England during 2006/07 (Defra 2009).

MSW	Estimated Composition (%)	Biodegradable Municipal Waste content (%)
Food waste	17,84	17,84
Garden waste	14,08	14,08
Other organic	1,73	1,73
Paper	16,65	16,65
Card	6,04	6,04
Glass	6,04	0
Metals	4,30	0
Plastics	9,99	0
Textiles	2,83	1,415
Wood	3,73	3,73
WEEE	2,19	0
Hazardous	0,53	0
Sanitary	2,51	1,255
Furniture	1,34	0,67
Mattresses	0,25	0,125
Misc combustible	2,37	1,185
Misc non-combustible	2,82	0
Soil	0,18	0
Other wastes	2,32	1,16
Fines	1,66	0,83
Total	100	66,71

2.2.3 Biodegradable Municipal Solid Waste Management in EU-28.

The MSW management in EU-28 varies between different countries due to differences in waste composition, availability of land, costs, national preferences, material availability, energy recovery and expertise. MSW treatment approaches in EU-28 are given in Table 2-4 and Figure 2-2. Landfill (sanitary or uncontrolled) is the main treatment used for the 36.24% of the MSW produced; this is followed by recycling (24.45%); incineration (22.04%) and biological treatment (13.83%). Compared to other countered in Europe, Austria had the highest amount of MSW treated through biological processes; while Cyprus, Greece and Malta exhibited the

highest quantities of MSW deposited to landfill. The highest recycled waste rates are reported in Germany, Denmark and Switzerland. The countries which have the highest waste incineration rates are Denmark and Switzerland (Eurostat 2011).

Table 2-4: MSW treatment approaches in EU-28 for 2011 (Eurostat 2011).

Treatment	Kg/capita	%
Composting and Anaerobic Digestion	69	13.83
Incineration	110	22.04
Recycling	122	24.45
Deposit onto or into land	181	36.27
Other treatment	17	3.41

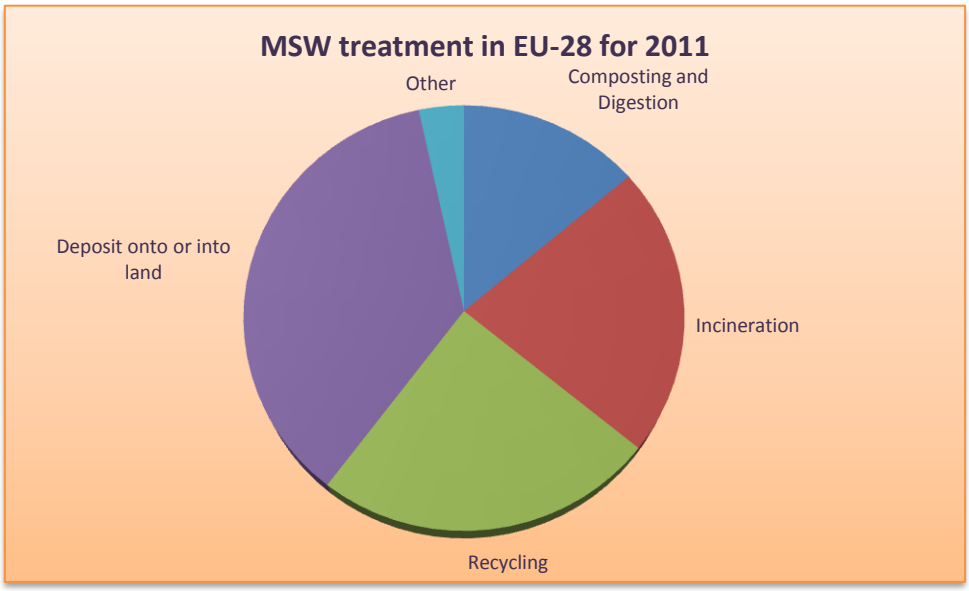

Fig 2-2. MSW treatment in EU-28 for the year 2001(Eurostat 2011).

2.2.3.1 Biological treatment: Composting and Anaerobic Digestion

Composting and anaerobic digestion are the two main processes used to treat the biodegradable fraction of MSW in Europe. Different process technologies have been used successfully for composting such as windrows, containers, box channels and tunnel composting. The duration of the process depends on the technique and varies from weeks to several months. The size of the composting plants varies depending upon the capacity. This can range from less than 1,000 tons to more than 100,000 tons per year. Composting benefits include the decrease of waste mass and volume; the recycling of nutrients; the carbon sequestration; the reduction of landfill related issues and the biodegradation of toxic compounds and other organic contaminants.

Anaerobic digestion is the degradation of organic matter under anaerobic conditions. The process is run in airtight reactors usually for several weeks, and produces biogas (CH4 and CO2) and digestate. Most of the anaerobic digestion plants convert biogas into heat and electricity. Others purify the biogas and inject it into the national gas grid or trade it as a transport fuel. The nutrients in the digestate waste produced can be recycled by applying the digestate to land. The main advantage of AD compared to composting is the positive energy balance. AD is a net energy producing process (100-150kWh per ton of input waste) whereas composting is an energy consuming process (Hartmann and Ahring 2006). The biodegradability of the waste and hence the biogas potential of the waste substrate depends on the content of carbohydrates, lipids and proteins and cellulose. Thermophilic wet process operation using food waste gives the higher biogas yield which decreases with increasing amounts of yard waste or low quality paper (Hartmann and Ahring 2006).

2.2.3.2 Landfill

Landfill is defined as a waste disposal site for the deposit of waste onto or into land (i.e. underground) including internal waste disposal sites (i.e. where the producer is carrying out its own waste disposal at the place of production) and a permanent site (i.e. more than one year) which is used for storage of waste (EU 1999). It was the main treatment technology in the past and it has continued to be for many countries around the world. From open dumps to sanitary and controlled landfill, it has resulted in the long lasting accumulation of waste in the environment. Nowadays, landfill is generally recognised as the final destination for the refuse that cannot be further segregated or recovered in any other way. Specific criteria should be taken into account when planning a landfill site such as the distance of the site to the residential areas; the presence and height of ground water tables; the proximity of coastal waters, nature protection zones and archaeological sites; the geological and hydrogeological conditions of the area and the risk of flooding or landslides.

The percentage of waste disposed in landfill sites differs between the member states of EU-28. The EU Landfill Directive provides a good framework for countries to reduce landfilling of biodegradable municipal waste (EU 1999). All member states landfill a percentage of waste under specific conditions, although it is observed that some member states continue to landfill a significant amount of waste in uncontrolled sites.

Gas and leachate are the two main emissions from landfill sites. Landfill gas (CH_4 and CO_2 primarily) is generated by the anaerobic degradation of organic waste. Uncontrolled gas emissions are associated with greenhouse gas emissions, fire and explosion hazards and bad odours. Landfill leachate is generated by the process of rain water filtering through the decomposing waste and as such extracting soluble matter present in the waste. This can result in the leaching of contaminants present in the landfill site. Leachate, if untreated, endangers the quality groundwater and/or surface water.

2.2.3.3 Recycling

Recycling contributes to the decrease of the negative environmental impact caused by resource use and constitutes one of the objectives of EU thematic strategy on waste. Packaging waste, end-of-life vehicles, wood, construction and demolition waste, car tyres, batteries, electrical and electronic waste are widely recycled throughout Europe. The European Commission has issued specific directives for the recycling of these types of waste. The recycling rates usually depend on the price of the materials in the market, where copper and aluminium tend to be the most popular recycling materials. Packaging waste is a major fraction of MSW, especially in developed countries, because those products have short lifetimes. This fraction of waste is usually converted to refused derived fuel (RDF).

Recycling facilities affect the quantities of waste entering into other management and treatment systems. It helps to reduce the waste that ends up in landfill sites, provides the industry with essential supplies such as metals, paper, glass and plastic and saves energy as the energy needed for recycling materials is usually less than the energy needed for raw materials production.

2.2.3.4 Thermal treatment

Thermal treatment technologies have developed in the last few decades to an extensive waste to energy process. Incineration, pyrolysis and gasification are the main processes used for thermal treatment.

Waste incineration is the thermal conversion of waste with a surplus of air. The combustible components react with oxygen in the air, releasing a significant amount of hot combustion gas. The moisture content of the waste is evaporated in the initial stage of the incineration process. The combustion gas passes from the furnace to the

afterburner to ensure complete burnout. The main part of the ash content of the waste is collected as bottom ash while the rest (fly ash) is collected by using the appropriate cleaning systems (electrostatic filters or bag filters). Waste incineration plants are designed to treat waste with great variation in composition. The lower heating value of the waste is the most important variable for determining whether the waste can support the combustion process without supplementary fuel.

Many countries consider incineration as a safe waste treatment method, reducing fossil fuel consumption. Other countries consider incineration as a major contributor to air pollution and a risk to public health. A major benefit of incineration is the possibility to recover the energy from the waste. The energy can be recovered as hot water and subsequently used for either district heating; process steam for various types of industries; electricity or for combined heat and power supply. The choice depends on the existing infrastructure for energy distribution; the price of the various types of available energy and the annual energy consumption.

Pyrolysis is the thermal degradation of organic material in the absence of oxidising materials such as oxygen, carbon dioxide or steam at temperatures which vary between 300-800^{O}C. During pyrolysis the pores of the solid waste are enlarged. This increases the porosity of the waste and thus the availability of sites for reactions with volatile and gaseous products to take place, favouring their interactions with the hot solid (Babu 2008). The pyrolysis process can be divided into conventional or carbonisation (slow heating rate), fast (fast heating rate, high temperatures, short contact time and very fine particles) and flash pyrolysis (high heating rate, small particle size). Fast pyrolysis of biomass exhibits notable economic and technical advantages due to it's potential to transform lignocellullosic biomass into liquid bio-oil with applications in both the energy and chemical sectors (San Miguel, Makibar et al. 2012).

Gasification is the thermal and chemical conversion of carbon-based material into a mainly gaseous output (syngas) by partial oxidation with a gasification agent (air, steam or oxygen) at a temperature between 800-1,100^{o}C with air oxidation agent and up to 1,500^{o}C with oxygen. The main advantage of gasification is the possibility of combining the operation conditions (temperature and equivalence ratio) and the features of a specific reactor (fixed bed, rotary kiln, plasma reactor, etc.) to obtain a syngas suitable for different applications (Arena 2012). Syngas can be utilized as a

fuel gas or combusted in a conventional burner connected to a boiler and a steam turbine or used for the production of chemicals or fuels in chemical industry.

Technical issues with gasification are generally a result of the highly heterogeneous nature of the feedstock, for example, MSW, whilst economic issues generally relate to the high capital and operating costs compared to that of conventional incineration. Independently verified tests have shown that gasification is able to meet the existing emission limits (Arena 2012).

2.2.4 EU policy and legislation on waste

Improved waste management is an essential element in efforts to make Europe more resource efficient. Current European policy on waste management aims to achieve a shift up the waste hierarchy by reducing waste disposal and focusing on waste prevention, recycle, reuse and recovery (EU 2008).

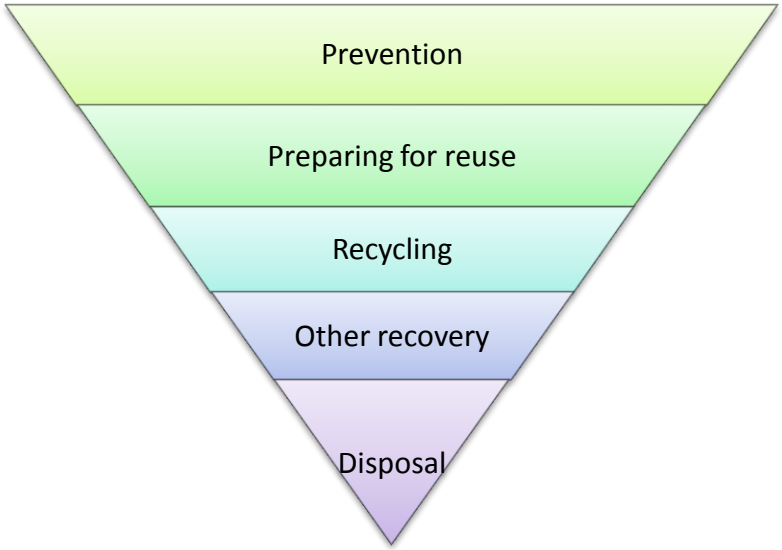

Fig 2-3. EU hierarchy on waste.

Waste prevention is the most efficient way to save resources and reduce the impact on the environment. Waste minimisation encompasses preventing and/or reducing the amount of waste at the source; improving the quality of the waste generated, such as reducing the hazardous element and encouraging reuse, recycling and recovery. The consensus understanding of waste prevention achieved by OECD countries can be divided into three types of actions: strict avoidance, reduction at source and product reuse (OECD 2000). The EU thematic strategy on waste describes a number of key objectives which intend to contribute towards the reduction of negative environmental

impacts as a result of resource use, in order to secure a higher level of environmental protection (EU 2008). These objectives are: to prevent waste; to promote reuse, recycling and recovery and to establish an attitude of recycling in society.

EU's waste framework directive (EU 2008) and landfill directive (EU 1999) set targets for recycling municipal waste and diverting biodegradable municipal waste from landfill. The recycling target for household wastes is set to 50% by 2020.

According to article 13 of the Directive 2008/98/EC on waste (EU 2008), the members states shall take the necessary measurements to ensure that waste management is carried out without endangering human health, without harming the environment and in particular: (a) without risk to water, air, soil, plants and animals, (b) without causing a nuisance through noise or odours and (c) without adversely affecting the countryside or places of special interest.

In order to achieve these objectives it was proposed to take forward the actions intended to modernise the legal framework related to waste management. These efforts included introducing life-cycle-analysis into policy-making frameworks and clarifying and simplifying law on waste.

Implementation of EU policy on waste shows large differences in the EU member states. Differences can be identified between the waste treatments being used; the status and development of the recycling facilities; the compliance with the EU directive on waste; the existence and application of economic instruments to support waste management; the adequate network of waste treatment facilities and the quality of future planning proposals.

Waste management policies have been constrained by a series of failures and barriers (Pearce and Turner 1993). These failures and barriers vary between different countries including: (a) information failure due to deficient databases on waste generation and disposal, (b) lack of "systems thinking" leading often to high incoherence, (c) lack of economic cost-benefit thinking leading to insufficient evaluation of the total treatment cost in social terms, (d) market failure due to the inefficient resource allocation because prices fail to reflect the full social cost, (e) lack of environmental awareness and sensitivity towards waste generation and other environmental implications, due to actions at the household, industrial or governmental level.

2.2.5 Final remarks

The waste management of MSW differs between European countries and depends mainly on national preferences, cost and energy recovery. Waste disposal to landfill is dominant followed by recycling, incineration and biological treatment. EU policy on waste aims to shift up the waste hierarchy by reducing waste disposal and increasing waste prevention and minimisation. Composting and anaerobic digestion are the two main processes used to treat the biodegradable fraction of MSW and divert this waste fraction from disposal in landfill sites.

2.3 Composting of Biodegradable Municipal Solid Waste

2.3.1 Introduction

The optimisation of economic, social and environmental benefits is essential to achieve sustainable waste management. It is critical that each component (economic, social and environmental) be given equal attention in order to ensure a sustainable outcome. The cost of composting varies depending on whether the process is carried out in-vessel or in open windrows; the pollution control equipment and the separation technologies. The analysis of the external costs and benefits of waste management treatments in Europe is favourable to the separate collection and treatment of biodegradable waste through composting or anaerobic digestion (Eunomia 2002). The costs of composting are likely to be lower under mandatory separate collections. Furthermore, costs of landfill and incineration have shown a tendency to rise (i.e. due to pollution control equipment), whereas the costs of composting and anaerobic digestion have shown a tendency to fall (Eunomia 2002). The environmental benefits of composting include the decrease of waste mass and volume; the recycling of nutrients; carbon sequestration; the reduction of landfill related issues and the biodegradation of toxic compounds and other organic contaminants. Measuring social outcomes for waste management, such as community wellbeing and community cohesion, have been limited to descriptive expositions of assumed benefits and/or avoided costs (SITA 2012). Composting and especially on-site composting, is a well-known technology and the social benefits are widely known to the public. The

minimisation of odour production using in-vessel composting increases the social acceptance of the technology. Furthermore, the use of compost in arid land to restore soil enhances the available land use.

Composting can be regarded as the most sustainable technology, when compared to the other available waste disposal technologies, for the treatment of the organic fraction of MSW. Economic, social and environmental benefits can be optimised resulting in a sustainable outcome.

2.3.2 The process

Composting is a bioxidative microbial process leading to highly stabilised organic matter (OM), which may contribute directly to achieve soil conditioning and fertility (Bertoldi 1983). Under aerobic conditions, the degradation of OM is an exothermic process that produces energy in the form of heat, resulting in an increase in temperature and a high-temperature phase. Composting end products include carbon dioxide, water, minerals, and stabilized OM. The transformation of fresh OM into compost overcomes the phytotoxicity of non-stabilised OM; improves the hygienic nature of the material and produces a stable OM, rich in nutrients and C, which is known to be beneficial to both soils and plants (Ros, Klammer et al. 2006).

The starting material before composting must be physically and chemically conditioned to guarantee good process performance. Maximum process efficiency is required to produce high quality compost. The main factors needing to be optimised are oxygen supply, moisture content, temperature control, C/N ratio, pH, organic matter (quality and quantity), physical structure and balance of nutrients (Goberna 2010). The specific weight should be around 0.5 and water content around 60%. The structural strength of the material should permit interstices large enough to allow air to pass through the mass. The dimension of the particles should be between 3 to 5 cm, in order to allow degradation by the microorganisms present in the compost over a time period of no longer than 50 days.

The composting process is generally characterised by an initial short mesophilic phase (25-40^{o}C) and then a rapid transition to a thermophilic phase (35-65^{o}C). After the subsequent decrease in temperature, the maturation or compost stabilization stage starts (Tang, Atsushi Shibata et al. 2007). The length of the different composting phases depends on the nature of the OM being composted and the efficiency of the process, which is determined by several factors such as starting material, O_2 supply,

moisture content, active turning and outside temperature (Ryckeboer, Mergaert et al. 2003).

In the initial phase, energy rich, abundant and easily degradable compounds like sugars and proteins are degraded by fungi and bacteria that are generally referred to as primary decomposers. During this phase, fungi compete with bacteria for the easily available substrates. Since the maximum specific growth rates of bacteria exceed those of fungi, fungi are very soon out competed. Mesophilic organisms are inactivated by higher temperatures, and are, along with the remaining easily degradable substrates, eventually degraded by the succeeding thermophiles. The decomposition continuous to be fast and accelerates up to a temperature of about 62^{o}C. Thermophilic fungi grow at temperatures up to 55^{o}C, while higher temperatures usually inhibit fungal growth. A good supply of oxygen is more important for fungi than for bacteria, and even in force-aerated systems, temporary anoxic conditions may occur. For this reason fungi play a negligible role during the thermophilic phase, except for substrates rich in cellulose and lignin where fungi remain important degraders throughout the entire process. At temperatures under 60^{o}C, more than 40% of the solids are degraded within the first week. The temperature range between 50-65^{o}C is of selective advantage, particularly for the genus *Bacillus*. When the temperature exceeds 65^{o}C, *B. stearothermophilus* is often dominant. Also members of the *Thermous* group grow on organic substrate at temperature between 40 and 80^{o}C. A number of autotrophic bacteria (Hydrogenobacteria) have also been isolated from composts. These bacteria obtain their energy by the oxidisation of sulphur or hydrogen, and synthesize their organic matter from CO_2 (Beffa, Blanc et al. 1996b). Despite the destruction of most microorganisms above 65^{o}C, compost temperatures may exceed 80^{o}C. It is probable that this final temperature rise is not due to microbial activity, but rather to the effect of abiotic exothermic reactions in which temperature-stable enzymes of Actinobacteria might be involved. Such high temperatures are important for compost hygienisation, destroying human and plant pathogens, and killing weed seeds and insect larvae. The disadvantage of temperatures exceeding 70^{o}C is that most mesophiles are killed, and thus the recovery of the decomposer community is retarded after the temperature peak. When the activity of the thermophilic organisms ceases due to exhaustion of substrates, the temperature starts to decrease. Mesophilic organisms recolonise the substrate, either originating from the surviving spores, through the spread from protected micro niches, or from external

inoculation. While in the starting phase organisms with the ability to degrade sugars, oligosaccharides and proteins dominate, the second mesophilic phase is characterised by an increased number of organisms that degrade starch and cellulose. Cellulose degraders include the bacteria *Cellulomonas*, *Clostridium*, and *Nocardia* and Fungi of the genera *Aspergillus*, *Fusarium*, and *Paecilomyces* (Ryckeboer, Mergaert et al. 2003).

During the maturation phase, the quality of the substrates declines. The ratio of fungi to bacteria increases due to the competitive advantage of fungi under conditions of decreasing water potential and poorer substrate availability. Compounds that are unable to be further degraded, such as lignin-humus complexes, are thus formed and become predominant. Some authors have proposed a fifth phase, the curing phase or storage phase. In this phase physicochemical parameters do not change although it has been observed that changes in the microbial communities still occur (Danon, Franke-Whittle et al. 2008).

2.3.2.1 Composting parameters

Moisture content (MC) is one of the most important parameters in composting. The optimum MC varies with feedstock with typical values being between 60-70%. The system is becoming water-limited at MCs of less than 45%, whilst microbial activity is reduced at MCs of less than 35% on a weight basis. The minimum MC at which bacterial activity occurs is 12-15%. MC and losses are related to aeration and temperature because water is a product of aerobic respiration and evaporation is a function of exposure to air currents and temperature (Finstein and Morris 1975).

Temperature depends on how much heat is produced by microorganisms and how much is lost through aeration or surface cooling. A well-constructed compost system will heat up to 40-50^{o}C within the first 3 days. This can be an indicator that there are enough nutrients and moisture for rapid microbial growth.

The pH is usually acidic at the onset of decomposition and then rises, reflecting the loss of organic acids through volatilisation and mineral decomposition and the release of ammonia through mineralisation of organic nitrogen (Finstein and Morris 1975). The pH affects the potential of beneficial bacteria to colonise composts. Acceptable pH values should be within tolerable levels to microorganisms, i.e. bacteria generally need a pH range of 6-7.5; fungi can tolerate a wider range 5.5-8.0; and actinomycetes 5.0-9.0 (Boulter, Boland et al. 2000). It is also an important indicator of aeration

levels through the composting process. Well-aerated compost piles have a high pH, whereas piles with "pockets" of anaerobic conditions have decreased pH values (Jimenez and Garcia 1989). The pH values reached during the maturation are rather high and this fact can affect the fertility and productivity of soils depending on the compost type, as well as the development of pH sensitive plants (Boulter, Boland et al. 2000).

Electric Conductivity (EC) affects the quality of compost because it reflects the salinity and suitability of crop growth. EC is increased after the active phase of composting probably due to the release of soluble salts like ammonium and phosphate resulting from the decomposition of easily biodegradable organic substrates.

The decomposition rate and the use of the end-product (compost) depends on the carbon to nitrogen ratio. The initial ideal proportion of these two elements is about 30 parts carbon to 1 part nitrogen by weight. C/N ratio may need to be adjusted depending on the availability of these elements especially when high carbon materials are present. Carbon is lost as CO_2 during composting which narrows the C/N ratio. If nitrogen is limited to the system, the microbial population will not grow to its optimum size, and composting will slow down. If nitrogen is in excess, rapid microbial growth occurs, which accelerates the decomposition process. However this can create anaerobic conditions and produce bad odours due to ammonia losses, allowing valuable nitrogen to escape. The C/N ratio is an important factor in determining compost maturity and stability. An ideal C/N ratio of a mature compost is about 10:1, although sufficiently composted materials may fall within a range between 5:1 to 20:1 depending upon the feedstock (Mathur, Owen et al. 1993).

2.3.2.2 Carbon sequestration

Carbon sequestration in soils is a complex process including the humification of organic materials; aggregation by formation of organic-mineral complexes; deep placement of organic matter beneath the plough zone and deep rooting and calcification (Bruce, Frome et al. 1999).

Soil C pool comprises of soil organic carbon (SOC) and soil inorganic carbon (SIC) (Lal 2007). SOC includes humus and relatively inert charcoal C. SIC includes elemental C and carbonate minerals such as gypsum, calcite, dolomite, aragonite and siderite. The soil organic C is stored primarily in soil organic matter (SOM). Carbon sequestration into SOM has received recent attention due to the international concerns

about greenhouse gases and global warming (Swift 2001). It has been suggested that the sequestration of atmospheric CO_2 in SOM can contribute significantly to attempts to adhere to the Kyoto Protocol (Schlesinger 1999). The increase of SOC following application of compost is sequestered carbon, not contributing to greenhouse gases in the atmosphere (DEC 2006).

Land use, crop and soil management have drastic effects on the level of the soil organic carbon pool, and thus, C sequestration. Carbon sequestration can be increased by implementing practices such as improving fertility by adding inorganic and organic fertilizer amendments, increasing biomass, decreasing erosion etc. (Sparks 2003). Carbon binding to soil following compost application has been reported to be 9-14% depending on the soil type and crop rotation (Bruun, Hansen et al. 2006).

Carbon addition to soil can flow through various pools (active, slow and passive) and also can exit the system (i.e. as CO_2 or dissolved carbon). The passive pool of OM include very stable materials which remain in the soil for hundreds to thousands years. Because active pools have small resident times (less than 10 years) only the fraction of organic carbon which will remain in passive pools is considered as stored carbon for this study. The passive pool consists of 45-60% of the carbon in composts while 4-20% and 30-45% of the carbon in composts can be considered to be in the active and slow pool respectively (USEPA 2002). The net carbon storage value relies on three main input values: the direct carbon storage, the carbon stored resulting from HS formation, and the percentage of carbon in compost assumed to be passive, or resistant to degradation (USEPA 2012).

2.3.2.3 Available nitrogen and phosphorus

The application of compost and organic amendments in soil influence the soil OM and the soil nutrient status (Chang, Sommerfeldt et al. 1991). Nitrogen and phosphorus are essential macronutrients for plant growth.

The total available nitrogen in compost comprises of ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N). The ammoniacal nitrogen released through ammonification during composting can be immobilized as organic nitrogen, accumulate in compost, emitted as ammonia or converted into nitrate and then in molecular nitrogen through nitrification and denitrification. Nitrate and nitrite concentrations have been measured in composting materials and have found to be

low which results in low nitrification and denitrification rates (Hadas, Kautsky et al. 2004).

The availability of phosphorus in composts depends on the composting method and the feedstock. Phosphorus can be absorbed by plant roots only as soluble orthophosphate (H_2PO_{4-} and HPO_4^{2-}).

Aside from the positive impacts, the application of compost to soil may cause ecological damage such as accumulation of heavy metals or organic pollutants, or eutrophication due to the high levels of N and P contained in the compost. Groundwater pollution caused by leaching of NO_3^--N from agricultural systems has caused public concerns for decades. The U.S. EPA has established a maximum contaminant level of 10 mg/L nitrate as N for groundwater.

2.3.3 Emissions and energy consumption

Emissions are important in simple and open composting systems, such as windrows, where the emitted gases are not treated but only controlled. Microbial degradation of OM during composting results in gaseous emissions such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ammonia (NH_3) and carbon monoxide (CO) (Andersen, Boldrin et al. 2010). The unpleasant odours released during composting are mainly associated with emissions of NH_3 and volatile organic compounds (VOCs) such as terpenes, amines, alcohols, ketones and sulfur compounds (Maulini-Duran, Puyuelo et al. 2014). The composition of the waste, aeration, MC, mechanical agitation and temperature are the most important factors controlling emissions during composting. It has been estimated that composting contributes very little to national GHG inventories generating only 0.01-0.06% of global emissions (Amlinger, Peyr et al. 2008). Emissions during in-vessel composting have been reported to be 0.11 Kg NH_3, 0.36 Kg VOC, 0.075 Kg N_2O and 0.34 CH_4 per 1Mg of the MSW organic fraction (Colon, Cadena et al. 2012).

Energy consumption is highly dependent on the type of machinery used and the technology applied. Electricity is mainly used in in-vessel composting and aeration systems. Diesel is mainly used to handle the waste during treatment and in waste transportation. The energy inputs for electricity and diesel for in-vessel composting has been reported to be 871.90 MJ per Mg of MSW organic fraction (Colon, Cadena et al. 2012).

Water consumption is dependent on the technology used with in-vessel composting demanding higher quantities. The water consumption during composting has been reported to be 0-0.5 m^3 per Mg of MSW organic fraction (Colon, Cadena et al. 2012).

2.3.4 The product: compost stability and maturity

It is important to differentiate between compost stability and compost maturity. Compost stability reflects the degree of decomposition of the organic matter (Chen 2003). A material is considered unstable if it contains a high proportion of a biodegradable matter that may sustain high microbial activity. Compost stability refers to the level of activity of the microbial biomass and can be determined by the O_2 uptake rate, the CO_2 production rate or by the heat release by the microbial activity (Elwell, Keener et al. 1993). On the other hand, compost maturity is often characterised by germination indexes, which is a measure of phytotoxicity, by the degree of decomposition of phytotoxic organic substances produced during the active composting stage (Wu, Ma et al. 2000) or by nitrification (Bernal, Paredes et al. 1998) and has been associated with the degree of compost humification (Jouraiphy, Amir et al. 2005). A compost which is mature is also stable. Yet stable compost may not always be at a level of maturity adequate for use as a medium for growing a certain species of plant (McAdams 1996).

Recently, the Joint Research Centre of the European Commission published a series of reports called "End of Waste Criteria" including a separate report for compost and digestate. Criteria which has to be fulfilled by the compost in order for it to cease to be waste includes a minimum OM content (15%) or nutrient (N, P, K, Mg) content; maximum limits for heavy metals and persistent organic pollutants; minimum stability and limited content of pathogens (JRC 2014).

2.3.4.1 Compost stability evaluation

2.3.4.1.1 Physicochemical parameters

Physical parameters include temperature, odour and colour (Iglesias Giménez 2010). Temperature has been suggested to be one of the most convenient parameters for stability evaluation, but it is not always useful for monitoring biological activity and thus biological stability. Issues often range from the influence of other factors such as the pile size and weather conditions (McAdams 1996). The end-product after a sufficiently long period of maturation, has a dark-brown or almost black colour

(Iglesias Giménez 2010). The unpleasant odour of refuse decreases during the first stages of bio-oxidation and practically disappears by the end of the composting process. As odour and colour are two subjective parameters, these are not considered as accurate stability indicators. Physical characteristics give a general idea of the decomposition stage reached, but give little information with regards to the degree of maturation (Bernal, Paredes et al. 1998).

Chemical parameters such as pH, electrical conductivity (EC), cation exchange capacity, C/N ratio, thermal analyses and humification parameters have been applied as indicators of stability. Although pH is a parameter that is often determined it does not seem to offer any information on the course of the process, or the quality of the compost, because little variations of pH are observed. Also a pH around neutral is not sufficient to consider that the OM is stable. Generally, pH and EC may be used to monitor the compost stabilisation process, but only with a relatively consistent source of waste composition and calibration of other stability tests (Wu, Ma et al. 2000).

During efficient composting, C/N ratio is expected to decrease as a result of the degradation of organic matter and mineralisation (Harada, Inoko et al. 1981, Margesin, Cimadom et al. 2006). However C/N ratio changes irregularly with time. As the variation of the C/N ratio during composting depends on the type of compost, it is of limited usefulness. Some authors propose that a C/N ratio of less than 15 can be used as an indicator of OM stability, but in the case of sewage sludge the high nitrogen content leads to C/N ratios of below 15, despite the fact that the OM is fresh.

The cation exchange capacity increases as compost approaches stability and this increase is a function of humification due to the formation of carboxyl and phenolic functional groups (Lax, Roig et al. 1986). However, CEC of initial substrates vary and prevent the ability to define threshold values and to use it as a stability indicator.

Humification parameters, based on the fractionation of the extractable humic-like substances during compost maturation, are able to establish with accuracy the moment of complete stabilization (Ciavatta, Govi et al. 1990). Humification index (HI) and degree of humification allow the detection of different rates of organic matter transformation during the composting process.

Thermal analyses are based on programmed heating of samples in a controlled atmosphere, which finally provides qualitative and quantitative information regarding the compost humic matter content. Different scanning calorimetry (DSC) curves allow distinction between well and poorly stabilised OM(Pietro and Paola 2004).

Water Soluble Carbon (WSC) is also a suitable parameter used to indicate the degree of compost stabilisation. The water soluble carbon to nitrogen ratio (WSC/N) can be used as a parameter to indicate the stability of the tested materials (Castaldi, Alberti et al. 2005).

2.3.4.1.2 Biological parameters

Biological parameters like oxygen and CO_2 respirometry and enzyme activities have been proposed to measure compost stability. Respiration (CO_2 evolution rate and/or O_2 uptake) can be considered as a general measure of microbial activity and has been widely used to evaluate the stability of the compost (Lasaridi and Stentiford 1998a, Lasaridi and Stentiford 1998b, Gomez, Lima et al. 2006, Scaglia, Erriquens et al. 2007). The basis of these methods is that immature compost has a strong demand for O_2 and high CO_2 production rate due to the intense development of microorganisms as a consequence of the easily biodegradable compounds in the raw material.

CO_2 evolution is the most direct technique of compost stability because it measures carbon derived directly from the compost being tested (Kalamdhad, Pasha et al. 2008). CO_2 production is directly correlated with the aerobic respiration (Gomez, Lima et al. 2006). However monitoring of CO_2 evolution presents two major drawbacks; (a) CO_2 is soluble in aqueous solutions and (2) this solubility is pH dependent (Gomez, Lima et al. 2006).

Methods based on O_2 uptake rate (OUR) have been classified into dynamic and static. Dynamic methods use a continuous supply of air to minimize O_2 diffusion limitations (Gomez, Lima et al. 2006). Static methods do not allow the oxygen to be dispersed throughout the biomass and therefore it is possible to under-estimate the oxygen uptake (Adani, Lozzi et al. 2001). The problem can be solved by continuous biomass stirring and periodic aeration to replenish the oxygen consumed by the microorganisms (specific oxygen uptake rates or SOUR) (Lasaridi and Stentiford 1998a). The SOUR is a simple technique used to assess compost stability (Lasaridi and Stentiford 1998a, Gazi, Kyriacou et al. 2007). Another way to solve the problem of underestimating oxygen uptake by the static methods is the continuous aeration of the biomass. The dynamic respiration index (DRI) represents the reproduction of the full-scale process using a laboratory approach (Adani, Ubbiali et al. 2006) and determines the biological stability of the compost because it is able to measure the easily degradable organic fraction (Baffi, Dell'Abate et al. 2007). Two DRIs are

defined depending on the operating conditions: (a) the real dynamic respiration index (RDRI) and (b) the potential dynamic respiration index (PDRI), without or with adjustment to optimal moisture content respectively (Adani, Lozzi et al. 2001). The static respiration index (SRI) is determined using the same scientific apparatus as that used for determining the DRI. The oxygen uptake rate is ascertained by following the changes in oxygen concentration with time, in the air-space on top of the solid sample in the sealed container, after which aeration is stopped (Adani, Lozzi et al. 2001).

An alternative to aerobic respirometric methods is the Dewar self-heating test, which measures the heat produced by the sample under aerobic conditions, rather than the gases consumed or produced (Wagland and Tyrrel 2010). The principle of the method is to precisely record the highest temperature achieved after placement of compost into the vessel for several days (Brinton, Evans et al. 1995). The rate of microbial respiration affects the heat input which is reflected in the rise in temperature. The higher the temperature reaches, the less stable the material is (M.A. Sánchez-Monedero 2005). The self-heating test is widely adopted at solid waste plants in Europe to determine the biological stability of the compost produced. It is a good test and a good indicator of compost OM stability and does not require sophisticated equipment (Francou, Poitrenaud et al. 2005).

The Solvita maturity index provides a simple, relatively inexpensive test of compost stability and NH_3 emission for diverse samples of compost. Solvita measures CO_2 evolution and ammonia emissions simultaneously (Changa, Wang et al. 2003). Enzymatic activities have been proposed as a simple, quick and cheap measure for compost stabilisation (Mondini, Fornasier et al. 2004, Tiquia 2005). Enzymes play a role during composting as they are implicated in the biological and biochemical processes. Enzymatic activities are suitable as indicators of the state and evolution of organic matter during composting (Benitez, Nogales et al. 1999, Castaldi, Garau et al. 2008) and as stated earlier the stability of the end-product (Mondini, Fornasier et al. 2004, Gomez-Brandon, Lazcano et al. 2008).

2.3.4.1.3 Spectroscopic methods

Nuclear magnetic resonance of carbon is a valid tool for examining the chemical structure of natural OM and the chemical changes associated with OM decomposition. Changes can be measured on the bulk OM either fresh or composted, on humic substances extracted from the compost or on dissolved OM (Chen 2003).

Fourier transformed-infrared spectroscopy (FT-IR) is a technique that is efficient in providing comprehensive information on chemical composition of heterogonous materials (Wagland and Tyrrel 2010). FT-IR characterizes the principal classes of chemical groups that compose the organic matter (Chen 2003).

Excitation – Emission Matrix technology has been used to investigate organic matter dynamic in composting. The formation of HS can be monitored and provides evidence of progressive mineralisation and humification of the composting mixture (Antizar-Ladislao, Lopez-Real et al. 2006).

2.3.4.2 Microorganisms and enzymes

2.3.4.2.1 Microorganisms

A large variety of meshophilic, thermotolerant and thermophilic aerobic microorganisms (including bacteria, actinomycetes, yeasts, molds and various other fungi) have been extensively reported in composting and other self-heating organic materials at temperatures between 20-60^{o}C (Finstein and Morris 1975, Strom 1985, Amner, McCarthy et al. 1988, Faure and Deschamps 1991, Beffa 1996a). At an early stage of the composting process (20-40^{o}C) meshophilic/thermotolerant fungi, principally yeasts and molds, and acid producing bacteria are the dominant active degraders of fresh organic waste. Mesophilic organisms are partially killed or are poorly active during the initial thermogenic stage (40-60^{o}C), where the number and species diversity of thermophilic/thermotolerant bacteria, actinomycetes and fungi increase (Beffa 1996a). Enzymes play a role during composting as they are implicated in the biological and biochemical processes (Vuorinen 2000, Tiquia 2002, Ceustermans, Coosemans et al. 2010). Important enzymes during composting includes cellulases, β-glucosidaces, amidohydrolase, proteases, urease, phosphatases and arylsulphatase (Mondini, Fornasier et al. 2004, Ceustermans, Coosemans et al. 2010).

Compost microorganisms are influenced by the composition of the substrate and by the temperature in the compost pile. A major structural component of plants is lignin which may comprise up to 30% of wood. Lignin is primarily degraded by fungi that are often pathogens. Lignin degrading fungi are known as white-rot fungi. They degrade the lignin and leave behind the cellulosic components. Cellulose is the most abundant natural organic compound that is found in almost every type of organic waste. Fungi are, in general, more important for cellulose degradation than bacteria, which is especially the case if the cellulose is encrusted with lignin (e.g. in wood or

straw). Since cellulose is rich in C but does not contain N or other essential elements, the mycelial structure gives fungi a competitive advantage in cellulose degradation as nutrients may be transported in mycelium. Xylan is the most important of the three hemicellulosic compounds, and is found in straw, baggase (up to 30%) and wood (2-25%). The main degrading enzymes are xylanases, produced by many bacteria and fungi. Pectin is degraded by pectinases which are commonly produced by fungi and bacteria, many of them plant pathogens. Starch is composed of amylase (20%), unbranched chains of D-glucose and amylopectin.

Prokaryotes are the most abundant organisms on earth and comprise of two separate but related groups: the bacteria (or eubacteria) and the archaea (or archaebacteria). Their size ranges from 0.1 to 10 μm and have one of three basic shapes: spherical, rod-like or helically coiled. The bacteria are the commonly encountered prokaryotes in soil, water and living organisms and include Escherichia Coli, the Bacillus species and the Cyanobacteria. The archaea includes the sulfur reducing bacteria and the methanogens and are found in unusual environments such as salt brines, hot acid springs, bogs and the ocean depths (Hames 2005).

Most prokaryotes have an optimum temperature, oxygen concentration, pH and water activity for growth and survive in a range of conditions around these optima. Depending on the optimum temperature, prokaryotes classified as phychrophiles (optima 20^{o}C), mesophiles (15-45^{o}C, optima 37^{o}C), thermopiles (30-75^{o}C, optima 55^{o}C) and hyperthermophiles (optimum greater than 80^{o}C). Prokaryotes vary in their requirements for oxygen, depending on the nature of their metabolism. Aerobes are capable of growing in the presence of oxygen while anaerobes do not require oxygen for growth.

The rate of which prokaryotes grow depends on the nature of the microbe, the ingredients of the medium and the environmental conditions. Prokaryotic cells, under appropriate environmental conditions and sufficient ingredients, accumulate nutrients, synthesize new cell constituents, grow in size, replicate its genetic material, lay down new cell walls and eventually divide in two.

Bacteria can be classified as either Gram-positive or Gram-negative depending on whether or not they take up the Gram stain. To protect the cell from mechanical injury and osmotic pressure, bacteria are surrounded by a thick cell wall composed mainly of peptidoglycan (approximately 40%). Peptidoglycan is a complex of oligosaccharides and proteins and is responsible for strength, relative rigidity, shape and protective

properties of cell walls. Gram-positive bacteria (e.g. bacillus polymyxa) have a thick (25nm) cell wall surrounding their plasma membrane, whereas Gram-negative bacteria (e.g. Escherichia Coli) have a thinner (3nm) cell wall and a second outer membrane which is very permeable to the passage of large molecules (molecular weight >1000Da).

Fungi are heterotrophic, eukaryotic organisms with a filamentous, tubular structure, a single branch of which is called a hypha. The hypha extends by tip growth, and multiplies by branching, creating a fine network called a mycelium. Hyphae contain nuclei, mitochondria, ribosome, Gogli and membrane-bound vesicles within a plasma membrane bound cytoplasm. Not all fungi are multicellular, some are unicellular and are termed yeasts. Fungi are usually aerobic, but some species, like yeasts, are capable of living in low oxygen-tension environments and utilising fermentative pathways of metabolism. Phosphorus, potassium, magnesium, calcium and sulfur are all macronutrients required by fungi. Micronutrients include copper, manganese, sodium, zinc and molybdenum, all of which are usually available to excess in the environment (Nicklin J. 2002).

Fungi are the main agents of decay of cellulosic wastes produced by plants. The degradation that fungi perform with their extracellular enzymes is essential to the terrestrial biosphere. They decompose the waste material into CO_2, H_2O, and fungal biomass, which in its turn is decomposed by other microbes, returning mineral nutrients like phosphorus, nitrogen and potassium to the biosphere (mineralization process)(Nicklin J. 2002).

2.3.4.2.2 Enzymes

Enzymes are protein biocatalysts produced by living cells to bring about and control the numerous biochemical reactions involved in the metabolic processes of the cells. They differ from other catalysts mainly because of their protein nature. Being proteins, the enzymes are denatured and inactivated when subjected to abnormal conditions, such as heat or strong chemicals.

Enzymes function as specific catalysts which increase the rate of chemical reactions. Numerous factors affect enzyme activity such as time, the relative concentration of the enzyme and substrate, temperature, pH and the presence or absence of activators or inhibitors. The concentrations of substrate and enzymes are in excess in composting as well as time. Temperature affects enzymes in two ways. One effect is

thermal inactivation. High temperatures cause denaturation and unfolding of the enzyme protein, resulting in a loss of catalytic properties. There is a considerable variation in the actual temperatures at which heat inactivation is substantial, depending upon the particular enzyme. The second effect of temperature on enzymatic reactions is in their rate. A rise in temperature increases the rate of the chemical reaction up to the temperature in which thermal inactivation appears. A term frequently used is the "optimum temperature" which is the temperature for maximum velocity above which the rate decreases because of thermal inactivation. Each enzyme in the presence of its substrate has a characteristic pH (optimum pH) at which it is most active. The pH optima for different enzymes vary widely. When the pH of an enzyme-substrate system is changed, the enzyme's activity decreases. Certain anions, such as Na^{+}, Ca^{2+}, Mg^{2+} and Fe^{2+} (activators), are absolutely necessary for the activity of some enzymes whereas some others, such as Ag^{2+} and Hg^{2+} (inhibitors) are highly toxic.

An enzyme inhibitor is a substance which reduces or blocks enzymatic catalysis. Many poisons act by inhibiting a single enzyme in a major metabolic chain, thus having a profound and even fatal effect on the organism. Enzyme inhibition can be irreversible or reversible. If the enzyme activity is restored by removing the inhibitor, the inhibition is called reversible. Poisons or inhibitors, such as certain heavy metal ions, may result in irreversible inhibition by denaturation or destruction of the enzyme. Substances which combine with the enzyme at the same site as the normal substrate produce competitive inhibition. These substances usually have a structure similar to the normal substrate and block the access of it to the active centers. Non-competitive inhibitors are substances which combine with sites which are not essential for substrate binding, but which are essential for enzymatic action (Brinton M. Miller 1976).

2.3.4.3 Bacteria's resistance to the presence of inhibitors, desiccation tolerance and bacteria response to cold shock

As some metals react with anions such as chloride, phosphate, and hydroxyl ions to form insoluble precipitates, chemical interactions between metals and medium components may overestimate the resistance level of the microbe (Konopka, Zakharova et al. 1999, Murata, Kanao-Koshikawa et al. 2005). Silver has long been known to exhibit a strong toxicity to a wide range of microorganisms (Liau, Read et

al. 1997, Morones, Elechiguerra et al. 2005). The bactericidal effect of silver ions on microorganisms is very well known; however the bactericidal mechanism is only partially understood (Liau, Read et al. 1997, Morones, Elechiguerra et al. 2005). Morones et al. (2005) identified that silver nanoparticles (1-10nm) act primarily in three ways against gram-negative bacteria: (a) they attach to the surface of the cell membrane which reduces the membrane's permeability and ability to respirate, (b) they penetrate inside the bacteria and cause further damage possibly through their interaction with S and P containing compounds such as DNA and (c) the nanoparticles release silver ions which according to Feng et al. (2000) are able to penetrate the cell wall and enter inside the cell. This subsequently turns DNA into a condensed form, which at the same time reacts with proteins. All these phenomena lead to the damage and/or death of the microorganisms (Feng, Wu et al. 2000). Guzman et al. (2012) identified that the Ag-nanoparticle size has an effect on microbes: as the nanoparticle's size decreases, the minimum inhibitory concentration decreases for bacteria (Guzman, Dille et al. 2012). Pal et al. (2007) provided evidence that silver nanoparticles undergo shape-dependent interaction with the gram-negative bacterium E. coli (Pal, Tak et al. 2007).

Resistance to dehydration is crucial to microbial life because it can damage DNA, denaturise proteins and increase the melting temperature of the cell membrane. The results in a transition to the gel phase at a temperature where the membrane should be in the liquid crystalline phase (Ramos, Gallegos et al. 2001). The bacteria's response to dehydration includes the synthesis and intracellular accumulation of disaccharide sucrose and especially, trehalose. Trehalose acts as an osmoprotectant in many organisms; in bacteria and yeasts it also increases their resistance to desiccation (Welsh and Herbert 1999). During desiccation reversible changes to yeast and bacteria's fatty acid composition are made, in order to preserve the cell's membrane. Halverson and Firestone (2000) identified that the length of the fatty acid chain, nor the level of fatty acid saturation of the *Pseudomonas putida* strain was not affected from a sudden decrease in water availability (Halverson and Firestone 2000). Rehydration following the long-term desiccation can be as stressful as dehydration itself. DNA damage incurred during desiccation has to be efficiently repaired, and its capacity seems to be crucial for survival after prolonged desiccation (Billi, Friedmann et al. 2000). The extent of DNA damage following desiccation is comparable to what

occurs when bacteria are exposed to high doses of gamma radiation (Mattimore and Battista 1996).

Bacterial cells exposure to low temperatures affects the membrane's fluidity, the protein flexibility and the nucleic acid conformation. For example, after a shift from their optimal growth temperature, at 37^{o}C, to a lower temperature at 15^{o}C, mid-log *E. coli* cells stop growing and the synthesis of most proteins is repressed (Ramos, Gallegos et al. 2001). Membrane fluidity decreases upon a downshift in temperature, affecting the membrane-associated cellular functions. The organisms overcome this by decreasing the degree of saturation in the membrane phospholipids to attain increased flexibility. Cold shock also causes a reduction in efficiency of translation transcription and DNA replication. Cold-shock proteins, such as GspA protein, are synthesised to overcome these deleterious effects. Phadtare et al. (1999) studied the response of *E. Coli* upon cold shock. This resulted in the cytoplasmic membrane, RNA/DNA and ribosomes acting as the cellular thermosensors and the major cold-shock protein GspA regulating the transcription, translation and mRNA stability (Phadtare, Alsina et al. 1999).

2.3.4.4 Humic substances formation

Organic matter in the environment can be divided into non-humic material (protein, polysacharides, nucleic acids, and small molecules such as sugars and amino acids) and humic substances (humic acids, fulvic acids and humins). The size, shape and molecular weight characteristics constitute major physical properties of the HS that have a profound effect on their interactions with other soil components (clay, metal ions and biota) and xenobiotics.

The colloidal range is regarded as extending from 0.001 to 1.0μm in diameter and represents an intermediate condition between true solutions (particles in ionic or molecular dimensions) and particulate suspensions (particles sufficiently enough large to settle under the influence of gravity). HA's fall within the colloidal size range. FA are also characterized as colloids despite of the fact that some low-molecular weight FA are too small to be classified as colloids. The term that describes a HS as a whole is a "macromolecule". Two general types of colloidal systems can be differentiated: hydrophobic and hydrophilic. Humic colloids fall into the latter category in that they have a high affinity for water and are solvated in aqueous solutions.

HS are polydispersed macromolecules and thereby exhibit a range of molecular weights from as low as a few hundreds for FA to as much as several hundred thousand for HA. It is difficult to characterize HA in terms of molecular mass and chemical composition due to their heterogeneity and the tendency to form colloidal aggregates.

Humins (HU) constitute the insoluble fraction of the HS at any pH value. HU have a marked colloidal character and are extremely important in the retention of pollutants in soils. They therefore may play a role in the mobilisation and bioavailability of pollutants and nutrients in soils (Alvarez-Puebla, Valenzuela-Calahorro et al. 2004). The accumulation of HU in nature slows down the mineralisation of OM because this heterogeneous mixture requires either a very large assembly of enzymes or very versatile enzymes in order to induce rapid OM mineralisation (Rice 2001).

According to Swift et al. (1979) the decomposition process can be divided into three stages: the primary decomposition stage feeds the secondary stage of decomposer growth and decay. Both the primary and secondary stages then support the third level which consists of humus formation (Swift 1979). The most important biochemical distinction is between the three types of metabolic pathway: that of carbon, that of nutrients (*e.g.* N) and that of aromatic compounds. The carbon path gives dynamic energy to the system. The aromatic pathway acts as the major resource quality factor by forming enzymatic recalcitrant molecules and also by the direct toxic effects of the phenolic or quinonic monomers. The sequestering of C, N and other nutrients into stable forms from which they are only able to release at very slow rates or under extreme changes of environmental conditions may be regarded as a type of chemical homeostasis.

2.3.4.4.1 The significance of organic matter (OM) in soils and environment

OM is beneficial for soils. The typical dark colour of many soils is caused by OM and may facilitate warming. OM can hold up to 20 times its weight in water so improves moisture retaining properties of sandy soil and helps prevent drying and shrinking. The combination of OM with clay minerals forms soil aggregates leading to a stabilized structure. It forms stable complexes with Cu^{2+}, Mn^{2+}, Zn^{2+}, and other polyvalent cations enhancing the availability of micronutrients to higher plants. The sorption capacity of the soil for a variety of organic and inorganic gases is strongly influenced by OM. The ability of the soil to function as a ‘sink’ for N and S oxides in

the atmosphere may be due in part to reactions involving organic colloids. It works as a buffer in slightly acid, neutral and alkaline ranges. It is a source of nutrients (CO_2, NH^{4+}, NO^{3-}, PO_4^{3-}, and SO_4^{2-}) for plant growth derived from the decomposition of organic matter (Stevenson 1994).

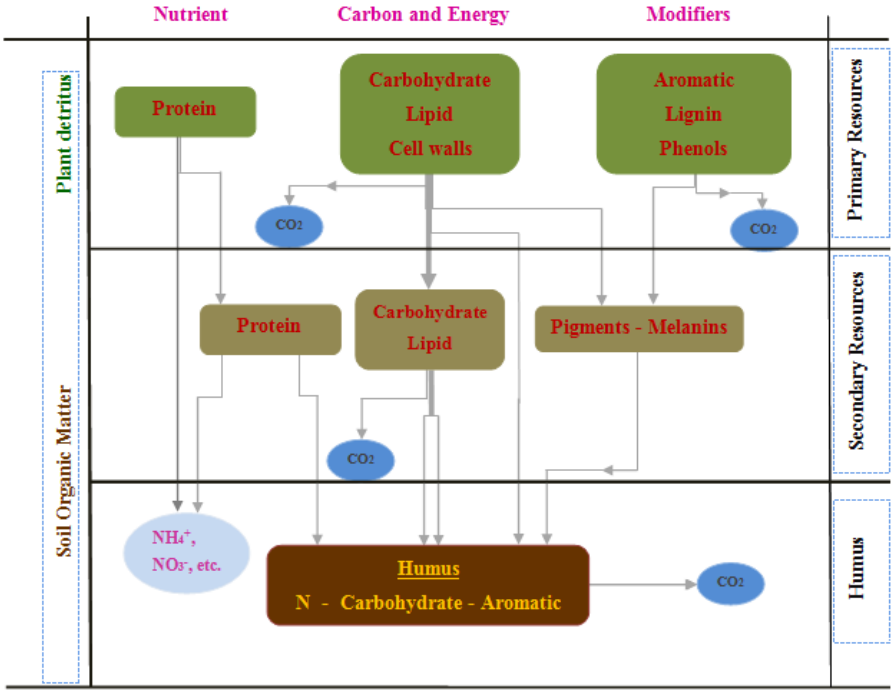

Fig 2-4. Major chemical pathways of decomposition processes (Swift 1979).

OM is important in both geochemistry and the environment. It may be involved in the transportation and subsequent concentration of mineral substances. It usually serves as a carrier of organic xenobiotics (as well as trace elements) in natural waters. Humic substances play a role in reducing the toxicity of certain heavy metals (e.g. Cu^{2+}, and Al^{3+}) to aquatic organisms. HA act as oxidizers or reducing agents, depending on the environmental conditions. They may affect photochemical processes in natural waters, including photoalteration of xenobiotics. Humic substances have been shown to reduce Hg(II) to volatile Hg^{o} under natural pH conditions, thereby providing a potential pathway for the mobilization of Hg in the environment.

2.3.4.4.2 Humic Substances interactions with metals

The selectivity and strength metal binding by HA depends on the amount of metals available for binding and the amount of metal bound (Ross 1994). The major functional sites for metal bonding are oxygen containing ligands, including carboxyl,

phenol, alcohol and carbonyl groups. The complexation of metal bonds/ions depends on the electronic status of the ligand site; the associated aliphatic chains, aromatic rings and geometry of the functional site and the environment surrounding the complexing site (pH, ionic strength, metal species) (Ross 1994).

Crucial to understanding the interaction between HS and metals is the electrostatic double layer which forms around the molecule due to its high charge. The presence of the negative potential around a large polyelectrolyte will cause cations to be attracted towards the surface producing a higher concentration of metal ions at the surface of the molecule $[M^{z+}]_D$, than in the bulk $[M^{z+}]_{BULK}$.

The capacity to bind metal ions is one of the most important properties of the HS. This property is mainly attributed to the large content of oxygenated reactive functional groups including carboxylic, phenolic, alcoholic and enolic hydroxyl groups and carboxyl functionalities of various types. HS can either form soluble or insoluble complexes with metal ions depending on the pH value, the presence of salts (ionic strength effect) and the degree of saturation of binding sites. FA can form more soluble, bioavailable and mobile metal complexes than HA because of their lower molecular weight and higher content of acidic functional groups. Thus, FA can act as carriers of metal ions in soils while HA tend to immobilize and accumulate metal ions in soil (Plaza, Brunetti et al. 2006).

2.3.5 Final remarks

Composting can be characterised as a sustainable solution for the management of the biodegradable fraction of MSW. Composting parameters such as MC, pH, temperature and C/N ratio are crucial for the performance of the process. The evolution of the process leads to the formation of HS. Compost quality depends on its OM and nutrient content as well as on the presence of heavy metals or organic pollutants. Different techniques have been applied to assess the stability and maturity of compost. Among those techniques, the monitoring of HS formation using Excitation Emission Fluorescence spectroscopy has been applied successfully for the characterization of compost.

2.4 Emerging contaminants in Biodegradable Municipal Solid Waste

2.4.1 Introduction

Nanotechnologies are an important set of emerging technologies at a very early stage in their development (HMGoverment 2009). Nanotechnology is defined by The British Standards Institution (BSI) as the "D*esign, characterisation, production and application of structures, devices and systems by controlling shape and size in the nanoscale, which covers the size range from approximately 1nm to 100nm"* (UK 2010). There is an ever growing number of current applications of nanotechnology in health and fitness, home and garden, electronics and computers, food and beverage, automotive, appliances and goods for children (Woodrow 2011). The global market in nano-enabled products is expected to grow from $2.3 billion in 2007 to $81 billion in 2015 (HMGoverment 2009).

The unknown environmental risks and long-term health impacts of nanotechnology are preventing scientists and the public from fully supporting the advancement of nanotechnology (Scheufele, Corley et al. 2007). The quantities and forms of NMs released from consumer products should be determined in order to assess the environmental risks of nanotechnology (Benn and Westerhoff 2008). The impact of nanotechnology needs to be addressed before nano waste appears in the environment; and therefore before new nano products are introduced in the market (Bystrzejewska-Piotrowska, Golimowski et al. 2009). Many NPs used by industry contain heavy metals, thus the toxicity and bioaccumulation of metal NPs may become an important environmental issues (Bystrzejewska-Piotrowska, Golimowski et al. 2009).

A recent study comparing TiO_2, ZnO, Ag, carbon nanotubes and fullerene NPs showed that nano-TiO_2 and nano-ZnO were the most likely to enter the natural environment in the largest quantities. This reflects to the worldwide volume of the production of engineered NMs (Gottschalk, Sonderer et al. 2009). Although the uncertainty about NMs production is high, it has been suggested, that worldwide production of TiO_2 engineered NPs, is ca. 5000ton/year (Nowack, Mueller et al. 2009) and its production and consumption in Europe ca. 3400tn/year (Gottschalk, Sonderer et al. 2009).

2.4.2 Nanomaterials and Nanoparticles

2.4.2.1 Definitions

Nanomaterials (NMs) are defined as materials with one or more external dimensions or an internal structure at the nanoscale, which could exhibit novel characteristics compared to the same material without nanoscale features (SCENIHR 2005). The nanosize results in specific physicochemical characteristics that may differ from those of the bulk substance or particles of larger size. This effect is mainly attributed to the high surface area to volume ratio, which potentially results in high reactivity (Wijnhoven, Peijnenburg et al. 2009).

NMs are designed to achieve particular physicochemical properties that relate to the product application. The size and shape (i.e. tubes, spheres), as well as the structure, influence the physical and chemical properties of the NMs. Two particles made of the same material may have different physical and chemical properties and uses (Ju-Nam and Lead 2008).

In general, NMs can be categorized into carbon-based materials (i.e. carbon nanotubes and fullerenes), inorganic nanoparticles (NPs) based on metal oxides (i.e. titanium dioxide, zinc oxide, cerium oxide, iron oxide), metals (i.e. gold, silver) and quantum dots.

2.4.2.2 Natural, Anthropogenic and manufactured NPs

Natural NPs have existed in the environment since the beginning of earth's history. They are generated by a wide variety of geological and biological processes. Natural sources include volcanic dusts, natural waters, soils and sediments. Examples of natural NPs are fullerenes and carbon nanotubes of geogenic or pyrogenic origin and atmospheric aerosols such as sea salt (Nowack and Bucheli 2007). In soils, natural NPs include clays, organic matter, metal oxides, and other minerals that play an important role in biochemical processes (Klaine, Alvarez et al. 2008).

Anthropogenic NPs can either be formed as by-products of combustion or produced intentionally due to their unique properties. Combustion processes emit a wide variety of particles but only ultra-fine particles, such as the "soot" fraction of black carbon, falls under the definition of NPs. In the case of production, NPs are referred to as manufactured or engineered. Manufactured NPs can be synthesized by a huge range of procedures which can be grouped into either top-down or bottom-up processing methods.

In top-down methodology the NPs are produced directly from the bulk materials via the generation of isolated atoms using various techniques such as milling or attrition, pyrolysis, repeat quenching and photolithography. Bottom-up methods are based on the combination of atomic scale materials to produce nano scale materials using various techniques such as complex coacervation of two oppositely charged polyelectrolytes, co-precipitation and nano precipitation or solvent displacement (Teow, Asharani et al. 2011). Chemical agents (surfactants) are used to stabilize the NPs against coagulation or aggregation by conserving particle charge and by modifying the outer most layer of the particle.

2.4.2.3 Carbon-based NMs.

The most common carbon-based NMs, fullerenes and carbon nanotubes, are carbon anisotropies with structures in the nanometer scale.

Carbon nanotubes can be obtained using various techniques such as arc discharge; pyrolysis of the hydrocarbons over catalysts; laser and solar vaporization and electrolysis with different resulted morphologies such as straight, curled, branched, spiral, and helix-shaped structures (Dresselhaus and Terrones 2013). Carbon nanotubes (CNTs) can be classified as single-wall (SWCNT) or multi-walled (MWCNT). MWCNTs contain several SWCNTs in their structure. SWCNTs exhibit a strength to weight ratio 460 times that of steel. These structures were found to have excellent thermal and electrical conductivity.

Fullerenes are molecules with at least 60 atoms of carbon (C60). Carbon atoms in fullerenes form 12 pentagon rings and m hexagonal rings where m=(n-20)/2 and n is the number of carbon atoms in the molecule. C60 discovered in 1985 and it is the smallest fullerene that fulfils the isolated pentagon rule: "pentagons should be separated from each other by hexagons to avoid the inherent instability associated to fused pentagons" (Mojica, Alonso et al. 2013). Because of their electronic properties and their chemical reactivity, fullerenes are used widely in solar cell construction materials, medicine, nanosensors, antioxidants, etc.

2.4.2.4 Metal Oxide NPs.

Metal oxide NPs are extensively used in a considerable number of applications in food, material, chemical and biological science (Aitken, Chaudhry et al. 2006). Although the uncertainty about NMs production is high, it has been suggested that the

worldwide production of TiO_2 engineered nanoparticles, to be ca. 5000ton/year (Nowack, Mueller et al. 2009) and its production and consumption in Europe ca. 3400tn/year (Gottschalk, Sonderer et al. 2009).

2.4.2.4.1 Titanium Dioxide Nanoparticles

Titanium dioxide (TiO_2) or Titania exists in four main crystallographic structures in nature: anatase (tetragonal), rutile (tetragonal), brookite (orthorhombic) and TiO_2 (B) (monoclinic) (Gupta and Tripathi 2011). Each structure exhibits different properties and has different applications and environmental impacts. Rutile and anatase are the most important forms with regards to the environment.

A variety of methods are available for the synthesis of nano-TiO_2 (nanoparticles, thin films, nanoporous materials) including mechanical, chemical or physical methods; in a liquid or gas medium. Rutile is thermodynamically stable and it is mainly prepared using the hydrothermal approach (relatively high pressure and temperature). Anatase is metastable. The most widely used method for the synthesis of anatase is the hydrolysis of Ti(IV) under mild conditions (Brayner, Coradin et al. 2013).

TiO_2 is a semiconductor with a wide range of applications mainly due to its electronic and structural properties. TiO_2-NPs are mainly used as a white pigment (in paints, plastics, papers, foods and pharmaceuticals) and in sunscreen lotions to block UV-A and UV-B irradiation. They are characterised by chemical stability and the ability to degrade a wide range of both gaseous and liquid pollutants (Brayner, Coradin et al. 2013). They are used to coat glazing since they have both sterilising and anti-fouling properties. They are hydrophilic and because of this property they form sheets out of rain drops which then wash off the dirt particles broken down by the degradation process (Mann 2006). TiO_2-NPs and ZnO-NPs are used to degrade the paint additive izothiazoline-3-ones which is used to increase paint's shelf life by preventing the growth of moulds and fungus (Kandavelu, Kastien et al. 2004).

2.4.2.5 Metal NPs.

The most common used metal NPs are quantum dots, gold, silver and zero-valent iron NPs. Quantum dots are small assemblies of semiconductor materials (CdS, CdSe and CdTe) in the range of 2-10nm. They possess unique electronic, optical, magnetic and catalytic properties (Wang, Ruedas-Rama et al. 2007). One of the most important properties of metal NPs is the resonance effect or surface plasmon resonance (SPR)

which is caused by the interaction with the incident light and the free electrons in the materials (Noguez 2007). The resonance effect depends on a number of factors such as shape, surface chemistry, aggregation state and size. Aqueous solutions of gold nanoparticles citrate-stabilized are red colloidal solutions which present a surface plasmon band (SPB) at 550nm (Noguez 2007) whereas the corresponding AgNPs have an SPB at 414nm (Doty, Tshikhudo et al. 2005).

NPs are being incorporated into many products of daily use, e.g. fillers, opacifiers, catalysts, pharmaceuticals, lubricants, cosmetics, devices, or other domestic appliances (Bystrzejewska-Piotrowska, Golimowski et al. 2009).

2.4.2.5.1 Silver Nanoparticles

Currently, the most common method to synthesize spherical, monodisperse AgNPs is chemical reduction of silver salts and it is classified as a top-down methodology. This synthetic method involves the reduction of an ionic salt in an appropriate medium in the presence of a surfactant using various reducing agents such as sodium borohydride, hydrazine hydrate, sodium citrate and ascorbic acid (Guzman, Dille et al. 2012). Colloidal stability is critical for applications which require the dispersion of AgNPs in aqueous solutions. The high surface area to volume ratio results in high reactivity which leads to particle aggregation and settling. The enhancement of colloidal stability is achieved using capping agents which either enhance the surface charge or introduce steric or electrosteric repulsion (El Badawy, Luxton et al. 2010).

Silver's antimicrobial activity is the major driver for the development of AgNP products. Because of their significant antimicrobial properties, AgNPs are used in fabrics, cosmetics and have several medical uses (Ju-Nam and Lead 2008). Examples of AgNPs applications in consumer products include food packaging materials, food supplements, odour resistant textiles, electronics and household appliances, cosmetics, water disinfectants and room sprays. In medical applications AgNPs are used extensively for wound management, particularly for the treatment of burns, various ulcers, toxic epidermal necrolysis, for healing the donor sites and for meshed skin grafts (Wijnhoven, Peijnenburg et al. 2009).

2.4.3 Silver and titanium dioxide nanoparticles toxicity in the environment

2.4.3.1 Toxicity to microorganisms

A thorough understanding of NMs toxicity to microorganisms is important in order to evaluate the potential impact NMs will have on the environment as microorganisms provide key environmental services from primary productivity to nutrient recycling and waste decomposition (Klaine, Alvarez et al. 2008). Also, it is highly likely that microorganisms will influence the fate and behaviour of NMs in the environment.

Ge et *al.* (2014) studied the effects of TiO_2-NPs on soils bacterial communities. The results indicated that the presence of TiO_2-NPs altered the soil's bacterial community composition; but did not change selected soil properties such as soil water, soil organic matter, total carbon, total nitrogen and bioavailable carbon (Ge, Priester et al. 2013). In situ studies have demonstrated that silver, even in large particle form, inhibits microbial growth below concentrations of other heavy metals (Murata, Kanao-Koshikawa et al. 2005). Liang et al. (2010) studied the bacterial response to a shock load of AgNPs in an activated sludge treatment system. After the shock loading (0.75mg Ag / L), a prolonged period of nitrification inhibition along with ammonia and nitrite accumulation was observed. Microbial community structure analysis indicated that the ammonium-oxidizing bacteria and nitrite-oxidizing bacteria decreased (Liang, Das et al. 2010).

Peyrot et al. (2013) investigated the potential effects of AgNPs in soil by measuring the activity of specific enzymes in the presence and absence of organic matter. They found that AgNPs cause greater toxicity to soil enzymes at the low Ag concentrations while for higher concentrations the effect was less obvious. The presence of organic matter appeared to enhance the activity of the enzymes however the mechanism of organic matter action was not clear given that dissolved Ag concentrations were similar in both the presence and absence of organic matter in soil (Peyrot, Wilkinson et al. 2014). Kaegi et al. (2011) studied the behaviour of AgNP's in a pilot wastewater treatment plant. AgNP's sorb to wastewater biosolids and an extended AgNP's transformation into AgS_2 occurred. AgS_2 is a less soluble form and exhibits much lower toxicity than other forms of silver (Kaegi, Voegelin et al. 2011).

2.4.3.2 Toxicity to freshwater invertebrates

The effect of NMs in freshwater invertebrates has focused on a narrow range of test species, mainly on crustaceans, with *D. Magna* being the most studied test species.

Zhu et al. (2010) studied the toxicity and bioaccumulation of TiO_2 NPs aggregates in *D. Magna*. The experiments demonstrated a low toxicity of TiO_2-NPs even at concentrations as high as 100 mg/L in 48h tests. However, the NPs toxicity increased with the duration of exposure and inhibited the growth and reproduction of *D. Magna* and caused mortality even at concentrations of 0.5-5 mg/L (Zhu, Chang et al. 2010). Ribeiro et al. (2014) investigated the toxicity of AgNPs and $AgNO_3$ to three model organisms (*Pseudokirchneriella subcapitata, D. Magna and Danio rerio*) by assessing different biological endpoints and exposure periods. The study indicated that the effects of AgNPs in aquatic organisms are related to the particle characteristics, the growth media and the test species. They concluded that the toxicity of both AgNPs and $AgNO_3$ differ significantly based on these test species. There were no differences in toxicity for algae (*Pseudokirchneriella subcapitata)*, a small difference for zebrafish (*Danio rerio*) and a major difference in toxicity for *D. Magna* (Ribeiro, Gallego-Urrea et al. 2014).

Levard et al. (2013) studied the impact of sulfidation on AgNPs toxicity to four types of aquatic and terrestrial eukariotic organisms (zebrafish, killfish, least duckweed and nematode worm). The results showed that even partial sulfidation of AgNPs will decrease their toxicity because the concentration of silver ions decreases after sulfidation due to the lower solubility of Ag_2S compared to elemental silver Ag^0 (Levard, Hotze et al. 2013).

Muth-Kohne et al. (2013) examined the toxicity to AgNPs to zebrafish embryos before and after different sewage treatment plant (STP) processes. They observed an increased fish embryo toxicity of STP effluents with increasing AgNPs influent concentrations which identifies the accumulation of AgNPs in STPs as a potential source of effluent toxicity (Muth-Kohne, Sonnack et al. 2013).

Federici et al. (2007) studied the toxicity of TiO_2-NPs to the main bodily functions of rainbow trout after 2 weeks. No acute toxicity was observed in rainbow trout for concentrations up to 1 mg/L of TiO_2-NPs. However, they observed an unusual milky colouration of the luminal fluid and intestinal pathology. When these pathological results from the trout gut are considered, it seems probable that the nutritional performance of the gut will be affected. Also, the exposure to TiO_2-NPs caused respiratory toxicity and disturbances to the metabolism of some trace elements like Zn and Cu (Federici, Shaw et al. 2007).

2.4.3.3 Toxicity to marine organisms

Major differences are expected to exist in the chemical behaviour of NPs in seawater compared to fresh water that will have an impact on the fate and behaviour of NPs in such environments (Klaine, Alvarez et al. 2008).

Fabrega et al. (2008) investigated the impact of AgNPs on *Pseudomonas fluorescens* at different pH and in presence and absence of HA. They concluded that the presence of HA mitigated short term bacterial toxicity by forming a film on the NPs surface and thus changing the surface properties of the particles. Also they concluded that AgNPs toxicity is caused by their intrinsic properties and not by the dissolution of the NPs in suspension and subsequent effects of the dissolved Ag (Fabrega, Fawcett et al. 2009).

Miller et al. (2010) studied the impacts of TiO_2 and ZnO NPs on four species of marine phytoplankton. TiO_2-NPs showed no measurable effect on the growth rates of any species, while ZnO-NPs significantly depressed the growth rate of all four species (Miller, Lenihan et al. 2010).

2.4.3.4 Dissolution and toxicity of silver nanoparticles

The fate, transport and toxicity of NPs into the environment depends on the transformations that might be manifested in biological and environmental systems (e.g. dissolution and aggregation). The dissolution of AgNPs affects the bioavailability concentration in the environment and its assessment is critical for determining their toxicity (Harmon, Kennedy et al. 2014). The AgNPs release silver ions if oxidizing species like molecular oxygen or hydrogen peroxide are present (Loza, Diendorf et al. 2014).

Morones et al. (2005) studied the effect of AgNPs in the range of 1-100 nm on Gram-negative bacteria using scanning transmission electron microscopy. The results indicated that the bactericidal properties of silver nanoparticles are size dependent as the nanoparticles that have direct interaction with the bacteria have a diameter between 1-10 nm. They identified that AgNPs act primarily in three ways against Gram-negative bacteria. Firstly, AgNPs attached to the surface of the cell membrane disturbing its proper function; secondly they penetrate inside the bacteria causing further damage, possibly interacting with DNA and thirdly they release silver ions which have an additional contribution to the bactericidal effect of AgNPs (Morones, Elechiguerra et al. 2005).

2.4.4 Fate and concentration of NPs in the environment

The physicochemistry is essential to understanding the fate and behaviour of NPs in the environment, as well as uptake and distribution within organisms and the interactions of NPs with other pollutants (Handy, Owen et al. 2008). Complex interactions between abiotic and biotic components can have a significant effect on the fate of engineered NMs (Unrine, Colman et al. 2012). Adsorption of NOM on NPs can have dramatic impacts on particle dispersion resulting in altered fate, transport, bioavailability and toxicity (Gao, Powers et al. 2012). The presence of NOM in a AgNP solutions leads to a more stable AgNP suspension. This behaviour is due to the absorption of NOM molecules onto the surface of AgNPs and the resulting steric stabilisation of the particle suspension (Delay, Dolt et al. 2011).

Currently, data on background concentrations and physical-chemical form of NPs in the environment are scarce due to the limitations in separation and analytical methodologies. There is no available technique to quantitatively monitor NP emissions to and concentrations in the environment (Gottschalk and Nowack 2011). The majority of the available monitoring techniques are currently based on regulated substances that are either persistent, bioaccumulative and/or inherently toxic (Marcoux, Matias et al. 2013). Major pathways of engineered NPs to the environment include atmospheric emissions and solid or liquid waste streams (wastewater, sewage sludge) from production facilities. Coastal systems are likely to be the ultimate sink for any NPs discharged into the environment (Klaine, Alvarez et al. 2008).

The background concentration of a metal in soil and water is higher if the metal is abundant in the earth's crust. Silver is an extremely rare element in the earth's crust which means that background concentrations are extremely low. Titanium is the ninth most abundant element in the earth's crust so the background concentration is expected to be significant compared to the emitted concentration.

The predicted environmental concentrations in Europe for nano Ag, ZnO, TiO_2, CNT and fullerenes are given in Table 2-5. The higher concentrations for AgNPs and TiO_2-NPs are predicted to be 1.31-4.44 mg/Kg and 100-433 mg/Kg respectively for STP sludge.

2.4.5 Concentration of NPs in Biodegradable Municipal Solid Waste

For most consumer products the concentration of NPs in the product is unknown. The NP present in consumer products can be free or fixed inside the matrix of the product. It is not always clear how the NPs are fixed inside the matrix of the product and if they will stay fixed or migrate, evaporate, wash out etc. For example, a part of the AgNPs present in antimicrobial socks may wash out in the washing machine while another fraction may release when wearing the socks.

An estimation of the concentration of AgNPs and TiO_2-NPs in MSW can be made using their predicted concentrations in the sludge of sewage treatment plants (Table 2-5). AgNPs predicted concentration in STP sludge ranges between 1.31-4.44 mg Kg^{-1} for Europe whereas for TiO_2-NPs ranges between 100-433 mg Kg^{-1} (Gottschalk, Sonderer et al. 2009). NP concentration is expected to be higher in MSW because of the dry solid nature of the waste compared to the STP where due to the high water content of the sludge it is likely to be more dilute. An assumption can be made using the predicted concentrations of NPs presence in STP sludge as the lower concentrations predicted for MSW for both AgNPs and TiO_2-NPs.

Table 2-5: Predicted environmental NM concentrations shown as mode (most frequent value), and as range of the lower ($Q_{0.15}$) and upper ($Q_{0.85}$) quantities in Europe (Gottschalk, Sonderer et al. 2009).

Nano-TiO_2				
	Mode	**$Q_{0.15}$**	**$Q_{0.85}$**	
Soil	1.28	1.01	4.45	$\Delta\mu g\ kg^{-1}\ y^{-1}$
Sludge treated soil	89.2	70.6	310	$\Delta\mu g\ kg^{-1}\ y^{-1}$
Surface water	0.015	0.012	0.057	$\mu g\ L^{-1}$
STP effluent	3.47	2.50	10.8	$\mu g\ L^{-1}$
STP sludge	136	100	433	$mg\ kg^{-1}$
Sediment	358	273	1409	$\Delta\mu g\ kg^{-1}\ y^{-1}$
Air	<0.0005			$\mu g\ m^{-3}$
Nano-ZnO				
	Mode	**$Q_{0.15}$**	**$Q_{0.85}$**	
Soil	0.093	0.085	0.661	$\Delta\mu g\ kg^{-1}\ y^{-1}$
Sludge treated soil	3.25	2.98	23.1	$\Delta\mu g\ kg^{-1}\ y^{-1}$
Surface water	0.010	0.008	0.055	$\mu g\ L^{-1}$
STP effluent	0.432	0.340	1.42	$\mu g\ L^{-1}$
STP sludge	17.1	13.6	57.0	$mg\ kg^{-1}$
Sediment	2.90	2.65	51.7	$\Delta\mu g\ kg^{-1}\ y^{-1}$
Air	<0.0005			$\mu g\ m^{-3}$
Nano-Ag				
	Mode	**$Q_{0.15}$**	**$Q_{0.85}$**	
Soil	22.7	17.4	58.7	$\Delta ng\ kg^{-1}\ y^{-1}$
Sludge treated soil	1581	1209	4091	$\Delta ng\ kg^{-1}\ y^{-1}$
Surface water	0.764	0.588	2.16	$\mu g\ L^{-1}$
STP effluent	42.5	32.9	111	$\mu g\ L^{-1}$
STP sludge	1.68	1.31	4.44	$mg\ kg^{-1}$
Sediment	952	978	8593	$\Delta ng\ kg^{-1}\ y^{-1}$
Air	0.008	0.006	0.02	$\mu g\ m^{-3}$
CNT				
	Mode	**$Q_{0.15}$**	**$Q_{0.85}$**	
Soil	1.51	1.07	3.22	$\Delta ng\ kg^{-1}\ y^{-1}$
Sludge treated soil	73.6	52.1	157	$\Delta ng\ kg^{-1}\ y^{-1}$
Surface water	0.004	0.0035	0.021	$\mu g\ L^{-1}$
STP effluent	14.8	11.4	31.5	$\mu g\ L^{-1}$
STP sludge	0.062	0.047	0.129	$mg\ kg^{-1}$
Sediment	241	215	1321	$\Delta ng\ kg^{-1}\ y^{-1}$
Air	0.003	0.0025	0.007	$\mu g\ m^{-3}$
Fullerenes				
	Mode	**$Q_{0.15}$**	**$Q_{0.85}$**	
Soil	0.058	0.057	0.605	$\Delta ng\ kg^{-1}\ y^{-1}$
Sludge treated soil	2.2	2.1	22.2	$\Delta ng\ kg^{-1}\ y^{-1}$
Surface water	0.017	0.015	0.12	$\mu g\ L^{-1}$
STP effluent	5.2	4.23	26.4	$\mu g\ L^{-1}$
STP sludge	0.012	0.0088	0.055	$mg\ kg^{-1}$
Sediment	17.1	6.22	530	$\Delta ng\ kg^{-1}\ y^{-1}$
Air	<0.0005			$\mu g\ m^{-3}$

2.4.6 Legislation

There are regulatory gaps surrounding nanotechnology and NMs. The major problem is the lack of data on NMs health impacts, potential environmental toxicity, and the continuing inability to monitor any adverse effects. There is also a lack of coordinated, publicly available information about specific nano chemicals and materials, including where they are being produced and used, and what the potential risks may be (Senjen 2009).

There are many European Regulations and Directives that are relevant and applicable for the regulation of NM products but none of them are specific to the NMs themselves. For example, nanotechnology and NMs were not included in the scope of Regulation (EC) 1907/2006 (Registration, Evaluation, Authorization and Restriction of Chemicals, REACH)(EC 2006). However, there are on-going discussions on how REACH applies to NMs. In addition the new EU Regulation 1272/2008 on the classification, labelling and packaging of substances and mixtures that took effect on 20.1.2009 annulled the previous Dangerous Substances Directive 67/548/EEC and the Dangerous Preparation Directive 1999/45/EC (EC 2008). This new regulation is following the principles of the United Nations Globally Harmonized System (GHS) of Classification and Labelling of Chemicals. Although the regulations do not contain the terms nano and NMs under REACH and 1272/2008, manufacturer, importers and downstream users have to ensure that their NMs do not adversely affect human health or the environment (Kahru and Dubourguier 2010). The EU regulation No 528/2012 regarding the use of biocidal products introduces the term "nanomaterials" in EU legislation and defines NMs as "natural or manufactured active substances, in an unbound or bound stage or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size of 1-100 nm" (EU 2012).

2.4.7 Final remarks

There are a variety of NMs entering the market and a growing number of their applications in everyday life. The increased production and use of NMs introduces the NPs into the waste streams. This has increasing concerns regarding the environmental risks and long-term health impacts due to the research gaps in this field. Many studies are in progress including the toxicity of NPs and NMs in microorganisms, fresh water and marine organisms; but the research is in its early stage and the available data are

insufficient to result in acceptable conclusions. The regulation gaps in the field of nanotechnology can be attributed to this lack of sufficient information.

2.5 Impact of Emerging Contaminants on Composting and Compost

2.5.1 Introduction

The impact of NPs in composting can be assessed using EEM fluorescence spectroscopy. EEM fluorescence spectroscopy is a simple, low cost and reliable technique that has been successfully used to monitor the temporal dynamics of organic matter during composting (Antizar-Ladislao, Lopez-Real et al. 2006) and consequently the degree of compost maturity (Provenzano, de Oliveira et al. 2001). Fluorescence spectroscopy has been applied to the characterization and differentiation of HA and FA of different origins (McKnight, Boyer et al. 2001, Soler-Rovira, Brunetti et al. 2003) It has been widely used for the characterization of DOM in water and soil (Chen, Westerhoff et al. 2003).

2.5.2 Investigation of the Degree of Biodegradable Municipal Solid Waste Stabilization during Composting using Fluorescence Excitation-Emission Spectroscopy

The production of a 3D plot of fluorescence excitation wavelength, emission wavelength and intensity allows the visualisation of a range of fluorophores and their relative positions. Chromophores are defined as the molecules or moieties that absorb light and fluorophores as the fraction of chromophores that re-emit light at longer wavelengths (Mopper, Feng et al. 1996). An additional feature of excitation emission matrix fluorescence spectroscopy (EEMS) is the vast array of data available for interpretation within an EEM. Each EEM is a composite of emission scans from a single sample recorded at incrementing excitation wavelengths and arranged in a grid (excitation x emission x intensity). Attempts have been made to extend the understanding of fluorescence character and concentration by utilizing this large amount of data, for example by the analysis of the shape under the peak and the use of statistical techniques such as Analysis of Variance (ANOVA) and Parallel Factor Analysis (PARAFAC).

The wavelength at which absorption (excitation) and emission occur is specific to the molecule (Lacowicz 2006) and depends on the specific chemical structure as well as other factors including sample matrix and temperature. At low concentrations, measured peak intensity is directly proportional to the concentration of the responsible fluorophore in the solution (Henderson, Baker et al. 2009). Different fluorophores can have different efficiencies at absorbing and converting incident radiation to fluorescence. Quantitative and qualitative information can only be obtained from the changes in the intensity of a given component or from the ratios of any two components (Murphy, Stedmon et al. 2013).

Emission wavelength and relative intensity depends on the origin of HSs. The observed, distinct fluorescence properties of HSs provide useful diagnostic criteria for distinguishing between FA and HA from the same source as the molecular components of FA and HA differ with the source (Senesi, Miano et al. 1991). The groups of fluorophores identified by fluorescence are commonly named humic-like, fulvic-like and protein-like because their fluorescence occurs in the same area of optical space as the standards of these materials.

The emission intensity of aquatic dissolved organic matter increases with decreasing molecular size (Del Vecchio and Blough 2004, Boyle, Guerriero et al. 2009). The explanation is that intermolecular energy transfer, which competes with fluorescence and causes gradual energy dissipation, increases the concentration of energy acceptor-conjugated macromolecules. So, the lower molecular weight fractions are expected to be less subject to intermolecular energy transfer than higher ones and thus more fluorescent.

2.5.2.1 Fluorescence theory

The realisation that fluorescence was due to light absorption followed by light emission was first enunciated by George Gabriel Stokes (Stokes famous 1852 manuscript). Stokes observed that fluorescence is a longer wavelength than the excitation light (Stokes shift). Polarised fluorescence was observed later in 1920 by Weigert who wrote: "The degree of the polarisation increases with increasing molecular size, with increasing viscosity of the medium and with decreasing temperature, that is with the reduction of the mobility of the single particles".

The processes that occur between absorption and emission of light are usually illustrated by the Jablonski diagram (Fig 2-5). The absorption of light by a molecule

involves the transition of an electron from a low energy (ground, S_0) to a higher energy (excited, S_1) state. The relationship between the energy of the ground and the excited states is given by:

$$E = E_1 - E_0 = hv$$

where h is the Planck's constant and v is the frequency.

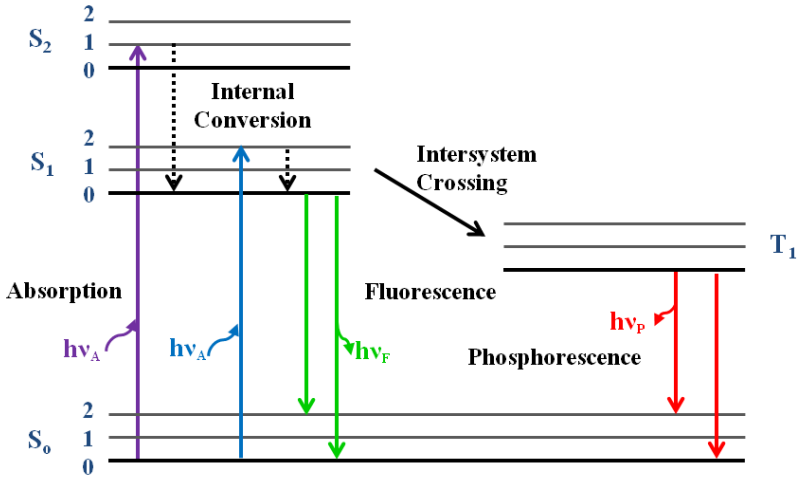

Fig 2-5. A typical Jablonski diagram (Lacowicz 2006).

Absorption is a very fast process, typically occurring within 10^{-15} seconds. Absorption occurs almost from the lowest vibration level of the ground state. At room temperature most of the molecules will be in this condition. After absorption, there is a rapid energy loss (either by vibration or by inter-system crossing) within the excited state which brings the excited electron down to the lowest vibration level and fluorescence occurs. Fluorescence emission occurs at higher wavelength than absorption because the energy involved in the fluorescence process is lower than in the absorption process.

Light consists of vertical and horizontal components. A polariser selectively passes light in one plane, so as this plane is in agreement with the reflected light, the reflected light can be seen. The direction of absorption and emission depend on the molecular orbital structure. Absorption and emission dipoles determine the orientation of light which the molecule absorbs, how strong this selectivity is and in which plane light is emitted. In a sample containing many molecules randomly orientated, only those which are correctly aligned are excited and emit a photon (as fluorescence). The emitted light is polarised in the same plane as the molecule's electric dipole. This phenomenon provides very useful information about the molecule's mobility within its environment. Polarisation and anisotropy are measured by exciting with plane

polarised light and measuring the emitted light parallel and perpendicular to the plane of the excitation light. Complex molecules with more than one excited state (i.e. those with S1, S2,etc. excited states) display wavelength dependent polarisation within the excitation spectrum.

2.5.2.2 Fluorescence parameters

2.5.2.2.1 Inner Filtering Effect

There is a lack of understanding of the effects of the inner filter, the absorption and re-emission of emitted energy at a longer wavelength by surrounding molecules during fluorescence analysis of dissolved organic matter, especially in concentrated solutions. The Inner Filtering Effect (IFE) refers to an apparent decrease in emission quantum yield and/or a distortion of bandshape as a result of the absorption of excited and emitted radiation by the sample matrix (Henderson, Baker et al. 2009). As a result for high concentrations of fluorophores in the solution there is a shift to longer emission wavelengths, and a shift to shorter emission wavelengths for low concentrations of solution. The potential for absorption of emitted light by surrounding fluorophores is reduced with the reduction of fluorophores present, so emission wavelength becomes a direct value with no associated energy loss to surrounding molecules (Hudson, Baker et al. 2007). Dilution of concentrated solutions is one of the methods suggested to eliminate inner-filter effect. Dilution range varies from x1 to x10 for treated sewage effluents, x10 to x100 for untreated sewage sludge and over x100 for some landfill leachates (Baker and Curry 2004). Absorbance is also used as a tool to determine if correction for IFE is needed. The threshold absorbance value will vary depending on the absorbance method and chromophoric organic matter present. For a 1cm cell, IFE is considered negligible for samples exhibiting absorption at or below 0.05 at a wavelength of 392nm (Kubista, Sjoback et al. 1994). Also, absorbance of samples in the UV-visible 0.1 or lower at 254nm has been suggested (Alberts and Takacs 2004). Many other authors have used a concentration of dissolved organic carbon (DOC) less than 10mg/L to eliminate the IFE.

2.5.2.2.2 pH effect

The wavelengths at which molecules fluoresce and the intensity of the fluorescence can be affected by a number of factors (except of the properties of the DOM) such as

changes in pH and temperature. The pH can affect the fluorescence intensity and the spectral shapes of humic and fulvic like substances.

2.5.2.2.3 Rayleigh and Raman scatter

Rayleigh and Raman scatters occur when a molecule has been excited to a virtual energy state by a photon with insufficient energy to completely excite the molecule. The virtual state is unstable and the molecule emits light. In a Rayleigh scatter the emitting light is of the same energy as the exciting light and as a result diagonal structure occurs in EEM at λem=λex. In a Raman scatter the emitting light is of a lower energy than the exciting light. The difference is dependent on the scattering molecule and it is called the "Stokes shift" (Larsson 2007 correction of inner filter effect). Both Rayleigh and Raman scatters can be removed from the EEM if during the processing of the data all the intensity values for emission wavelengths equal to or less than the excitation wavelength are replaced with a zero value. Rayleigh and Raman scatter using a UPW sample are given in Fig 2-6.

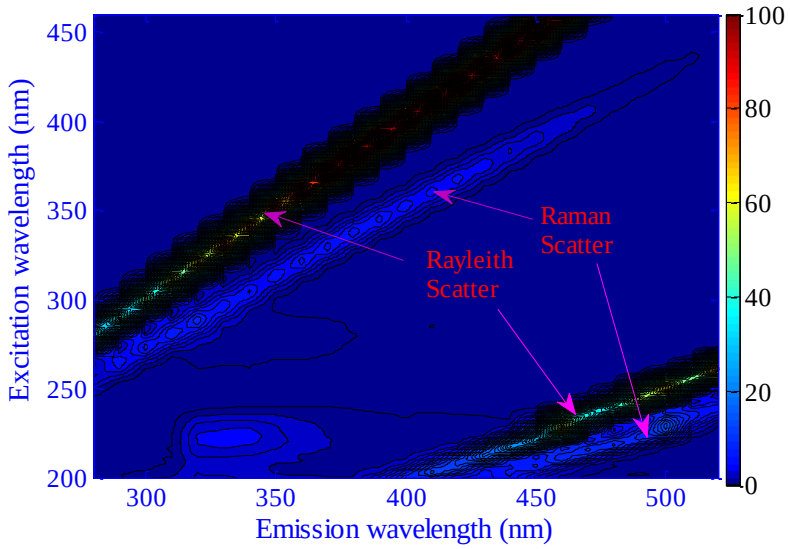

Fig 2-6. A Raman and Rayleith scatter using UPW sample.

2.5.2.3 Parafac Analysis

Parallel Factor Analysis (PARAFAC) is used to decompose trilinear multi-way data arrays and facilitate the identification and quantification of independent underlying signals, termed 'components'. It has been applied to a wide range of data including fluorescence excitation emission matrices (*sample* x *excitation wavelength* x *emission*

wavelength), chromatographic data (GC-MS: *sample* x *elution time* x *m/z stucture*), sensory data (*sample* x *attribute* x *judge*) and electroencephalography data (*space* x *time* x *frequency*).

PARAFAC decomposes the data into a set of trilinear terms and a residual array:

$$x_{ijk} = \sum_{f=1}^{F} \boldsymbol{a}if\boldsymbol{b}jf\boldsymbol{c}kf + \boldsymbol{e}ijk \qquad (1)$$

where i=1,...,I; j=1,...,J; k=1,...,K; x_{ijk} is the data point corresponding to the i^{th} sample at the j^{th} and k^{th} variable and e_{ijk} is the residual representing the variability not accounted for by the model. In fluorescence EEM, the i,j,k corresponds to the sample, the excitation wavelength and the emission wavelength respectively. Each f corresponds to a PARAFAC component.

The minimum number of trilinear (PARAFAC) components needed to reproduce an array is called the rank of the three-way array. Rank is not the same as column rank or row rank as it is in ordinary algebra where it is fundamentally a property that row rank equals column rank equals rank. For example, if you have an array with four components, the rank is 4.

The concentration of each component can be determined in each sample, if it is known that this component is attributed to a specific chemical analyte, by adding known quantities of the analyte (Murphy, Stedmon et al. 2013).

2.5.2.4 Excitation- Emission Matrix spectra of standard HA and FA

The EEM spectra of Leonardite HA standard, Pahokee Peat HA standard and Pahokee Peat FA standard from International Humic Substances Society (IHSS) are given in Fig 2-7. Two main peaks appear in the FA standard EEM spectra. This can be attributed to the variety of molecular weights that constitute FA. These two peaks may characterise two groups of the same molecular weight that are dominant in the solution. At least three main peaks appear in the HA standard EEM spectra at higher wavelengths compared to those of FA. The difference in the wavelengths can be attributed to the higher molecular weight and higher carbon content of HA compared to FA. The composition of the standards is given in Table 2-6. Also, the multiple peaks appearing in the HA EEM spectra may characterise multiple groups of the same molecular weight that are dominant in the solution.

The above observations result in the conclusion that the characterisation of FA and HA using EEM fluorescence spectroscopy is made using the multiple peaks that dominate in the respective solutions.

Table 2-6. IHSS standard FA and HA elemental composition.

Standard	Code	H_2O	Ash	C	H	O	N	S	P
Pahokee Peat Humic Acid	1S103H	11.1	1.12	56.37	3.82	37.34	3.69	0.71	0.03
Leonardite Humic Acid	1S104H	7.2	2.58	63.81	3.7	31.27	1.23	0.76	<0.01
Pahokee Peat Fulvic Acid	1S103F	9.3	0.90	51.31	3.53	43.32	2.34	0.76	<0.01

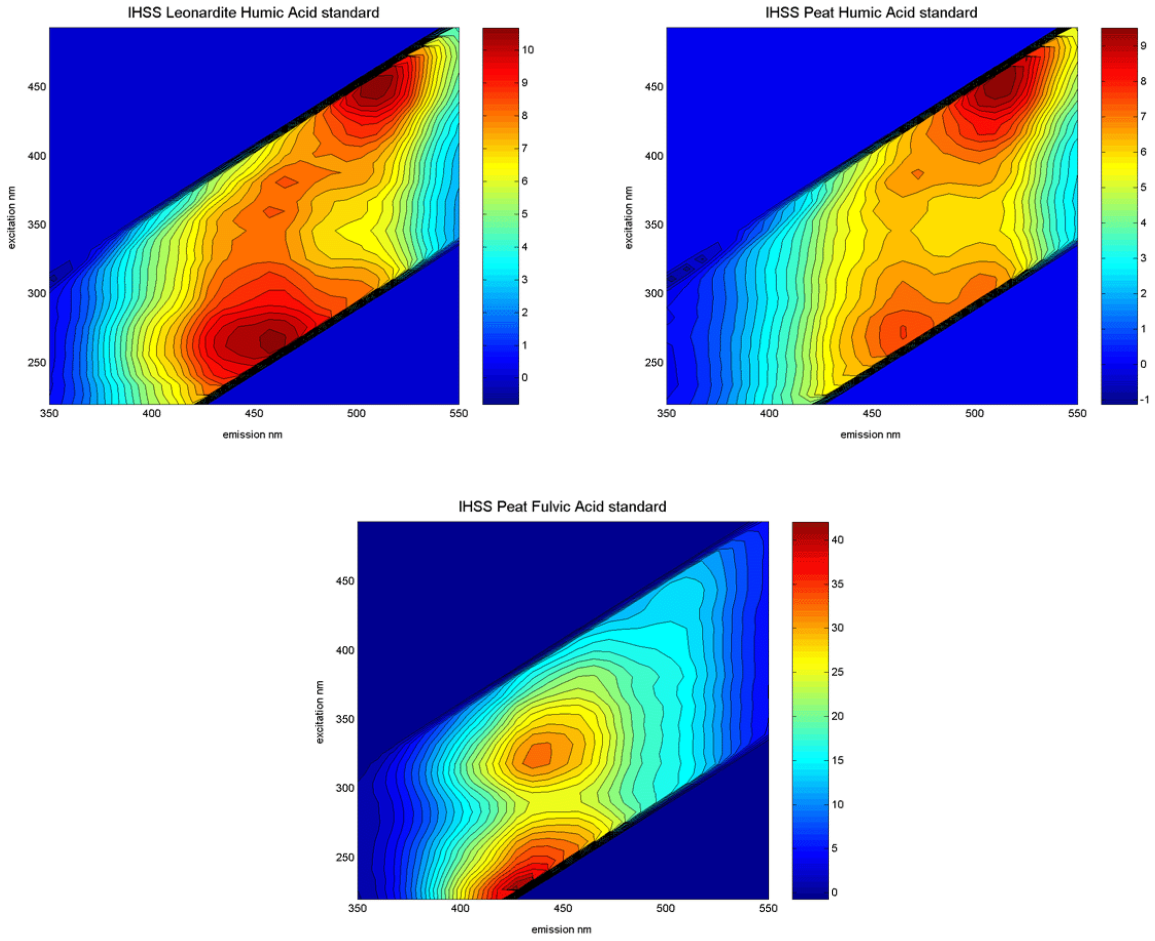

Fig 2-7. EEM spectra of IHSS Leonardite HA St, Pahokee Peat HA St and Pahokee Peat FA St (http://www.humicsubstances.org/spectra.html).

2.5.3 Final remarks

The impact of emerging contaminants, such as NPs, in composting can be successfully assessed by monitoring the formation of HS using EEM fluorescence spectroscopy. The formation of HS during composting can be used to determine the stability and maturity of compost. The IFE and pH are the main two parameters of fluorescence spectroscopy. Concentrated solutions can shift the EEM spectral peaks to higher wavelengths whereas pH affects the shape and intensity of the peaks. Multiple peaks that appear in FA and HA EEM spectra may characterise multiple groups of the same molecular weight that are dominant in the solution.

CHAPTER 3: EXPERIMENTAL RESEARCH

3.1 In-vessel Composting of NP-contaminated Biodegradable Municipal Solid Waste

3.1.1 Introduction

The main experimental research included the in-vessel composting of NP-contaminated biodegradable MSW. In-vessel composting is an industrial process used extensively to produce compost in a short period, usually 21 days, followed by the maturation phase lasting usually 2-3 months depending on the substrate. Several experiments using different concentrations of commercial NPs (Ag-TiO_2NPs and AgNPs) were conducted.

3.1.2 Aim and Objectives

The main aim of this study was to assess the impact of Ag-TiO_2NPs and AgNPs, at different concentrations of nano-Ag and nano-TiO_2, in the composting process.

The objectives of this study are as follow:

(a) Laboratory scale experiments to simulate an in-vessel composting process to investigate the effect of Ag-TiO_2NPs, at different concentrations, in the composting process,

(b) Laboratory scale experiments to simulate an in-vessel composting process to investigate the effect of AgNPs, at different concentrations, in the composting process,

3.1.3 Materials and Methods

3.1.3.1 Materials

3.1.3.1.1 Nanoparticles

Two commercial solutions of nanoparticles were used for the purpose of this research: (i) One commercial solution (photo catalyst coating) contained Ag-TiO_2NPs. It was a water-based, odourless, bluish white/yellowish transparent liquid. According to the manufacturer the average particle size was 8nm, the agglomeration index was between 2 and 4 and the crystallite structure of nano-TiO_2 was anatase. The matrix of the solution was unknown and confidential for the company. This type of NPs finds

applications in anti-bacterial and anti-virus fabric coating, air filter coating and home sanitization and as an additive of other anti-bacterial products; (ii) Another commercial solution of AgNPs (AgPURE W10) was supplied from RAS materials GmbH, Germany. The particle size of nano-Ag was 15nm according to the manufacturer. The matrix of the solution contained two stabilizers: 4% of PEG-25 glyceryl trioleate (PEG-25) and 4% of polysorbate 20 plus a small percentage of ammonium nitrate. This commercial solution was used as this type of NPs is recommended from the OECD for use in standardized ecotoxicological tests (Kerstin Hund-Rinke 2013). This aqueous nano-Ag dispersion presented an orange-brown colour. According to the supplier, it can be stored for 12 months in dark at a temperature range of 10-30^{o}C, and the NPs were used before the end of the recommended storage period. This commercial solution is used as an antimicrobial agent with applications ranging from varnishes and coatings over thermoplastic, duroplastic and elastomeric polymers to textile fibres.

3.1.3.1.2 Artificial Waste

Artificial biodegradable waste was prepared by mixing grass (20.5% d.w.), leaves (12.30% d.w.), wheat straw (23.04% d.w.), sawdust (39.30% d.w.) and foodstuff (equal amounts of cucumber, carrots, tomatoes, lettuce, onion and potatoes) (4.86% dw). Foodstuff and leaves were blended separately while grass and straw were cut using scissors. The composition of the green waste satisfied the nutrient requirement (C:N 40-50) according to the calculations using Cornell's system. The composition of the mixture is given in Table 3-1.

Table 3-1. Composition (%) of the composting mixture.

	w/w(g)	**MC (%)**	**d.w. (g)**	**%d.w.**
Grass	32	78	7.04	25.84
Leaves	16	72	4.48	16.44
Straw	5.8	7	5.39	19.80
Sawdust	10	8	9.20	33.76
Foodstuff	16.2	93	1.13	4.16
Total	**80**	**65.94**	**27.25**	**100**

3.1.3.2 In-vessel Composting Experimental Set-up

The effect of Ag-TiO_2NPs and AgNPs on the composting of biodegradable waste was investigated using laboratory-scale in-vessel composting reactors as in Antizar et *al.* 2006. Four tests were performed: biodegradable waste was composted; biodegradable waste contaminated with Ag-TiO_2NPs at different concentrations of nano-Ag [5, 10, 20 and 50 mg Ag/Kg OM (d.w.)] was composted; biodegradable waste contaminated with AgNPs at different concentrations of nano-Ag [5, 10, 20 and 50 mg Ag/Kg OM (d.w.)] was composted; and microbial activity was NaN_3-inhibited in the biodegradable waste reactors to evaluate abiotic losses.

The composting mixture was prepared by weighting the quantities of each component (Table 3-1) in wet basis (80 g) and mixing them together in a 2L beaker using a stainless steel spatula. The NPs were added to the mixture after dilution of the stock solution. The NPs were diluted at a concentration higher of 50 mg Ag / L to avoid potential NP-aggregation. UPW was added to the mixture to achieve the same initial moisture content in contaminated and non-contaminated reactors. The composting mixture of the NaN_3-inhibited experiment was prepared by adding 2% sodium NaN_3 to the mixture and mixing thoroughly. The composting reactors were vials 500 mL, but for NaN_3-inhibited experiments 250 mL reactors were used, mainly to reduce the amount of hazardous waste produced.

Composting reactors were placed vertically, in triplicates, in a temperature control incubator operated at a constant temperature, 38^{o}C, to simulate representative microbiological mesophilic stage during in vessel composting conditions (Fig 3-1, 3-2). Air was continuously flowing through the composting mixture, using an oil-free diaphragm pump, to avoid oxygen content limitation and the exhaust vented outdoors to avoid volatiles accumulation in the composting reactors. The air inlet was bubbled through a UPW reservoir to avoid excessive water evaporation during aeration.

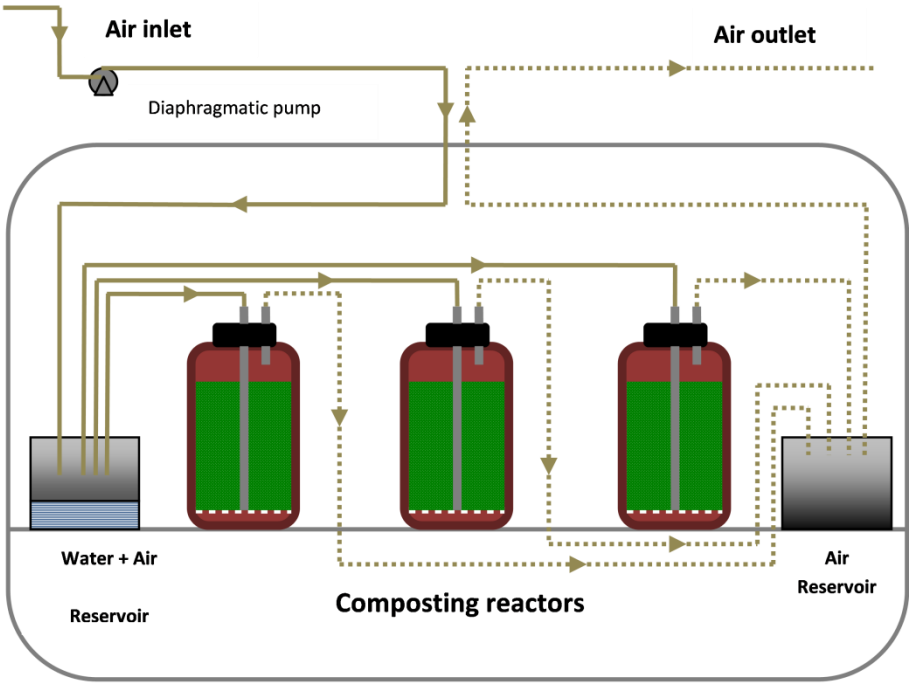

Fig. 3-1. Composting schematic experimental set-up(Adapted from Antizar et *al.* 2006).

Fig. 3-2. Composting laboratory experimental set-up.

The effect of initial AgNPs concentration in mg/Kg of organic matter (OM) on a dry basis was investigated in parallel (see Tables 3-2, 3-3). The control experiments include AT 0 and A 0 (composting without the addition of NPs) and the Matrix (composting with the addition of the matrix used for the production of NPs as described in section 3.1.3.1.1).

Table 3-2. Initial concentration values in experiments conducted with Ag-TiO_2NPs.

Experiment	AgNPs concentration	TiO_2-NPs concentration
	mg/Kg	mg/Kg
AT 0	0	0
AT 5	5	225
AT 10	10	450
AT 20	20	900
AT 50	50	2250

Table 3-3. Initial concentration values in experiments conducted with AgNPs.

Experiment	AgNPs concentration
	mg/Kg
A 0	0
A 5	5
A 10	10
A 20	20
A 50	50
Matrix	0

3.1.4 Analysis

Destructive sampling analysis was carried out in triplicates after 0, 4, 7, 14, 21 days. The moisture content, pH, ash content, % weight loss and the formation of HS were determined at each sampling day. The contents of each composting reactor were thoroughly mixed in a 2L beaker using a stainless steel spatula and sub samples were collected for analysis. One sample of each reactor was freeze dried for possible future analysis and kept in the freezer. The rest amount of the material was placed back in the reactors and kept in dark, at room temperature, for maturation. After maturation, for more than three months, the mature compost was grinded to pass a 2mm sieve and samples were analyzed to determine the total available nitrogen (ammoniacal nitrogen, nitrate nitrogen and nitrite nitrogen), available phosphorus and HS (HA and FA fraction).

3.1.4.1 Physicochemical Analysis

3.1.4.1.1 pH and EC

The pH and EC were measured at a ratio of 1:5 (w/w of compost to UPW) after filtration using Whatman filter paper No 2. The pH meter used was a Hanna pH meter model 991300.

3.1.4.1.2 Moisture Content, Ash Content, C/N ratio and weight loss.

Approximately 6 g (w/w) sample were dried at 105^{o}C for 24 h. The residual moisture of the samples was determined to produce the results on a dry matter basis. The moisture content of the NaN_3 samples followed a different protocol: the samples dried in the oven at 45^{o}C for 72h due to the NaN_3 presence. The validity of this suggested protocol was verified by drying green waste samples with identical MC at 45^{o}C for 72 h and at 105^{o}C for 24 h, which provided identical results of MC.

Ash content was determined using a loss-on-ignition procedure (Richard, Coelho et al. 2011). The dried samples transferred to a muffle furnace held at 550^{o}C for 4h. Ash content was calculated from the ratio of pre- and post-ignition sample weights.

C/N ratio was calculated by determining the total carbon (TC) and total nitrogen (TN) of the samples. TC and TN were determined using a NC Fisons Analyzer. The samples were dried, grinded using a coffee grinder and sieved (mesh 60) before analysis.

Weight loss was measured using the following formula:

$$\% \text{ weight loss} = 100 - [(W-W_v)/(W_o-W_v)x100]$$

where,

W = dry weight of vial and compost,

W_o = dry weight of waste at day 0 and

W_v = vial weight.

3.1.4.1.3 Available Nitrogen

The total available nitrogen comprises ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N). Ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) content were measured using automated colorimeter.

The sample analysis was conducted in the Crew Laboratory of the School of Geosciences at Edinburgh University. Ammonia was detected using Bran & Luebbe

continuous flow analyzer following Bran & Luebbe method G-102-93 Rev 1. Nitrate and nitrite detected using Bran &Luebbe continuous flow analyzer following Bran & Luebbe method G-109-94 Rev 3.

In this method, nitrogen in the samples reacts with reagents to form coloured samples. The stronger the colour formed, the more nitrogen is present in the sample. The strength of the colour is quantified by measuring the absorbance of light by the sample at specific wavelengths. The analysis protocol is given below:

1. Weigh 3g of compost.
2. Add 100mL of 6% w/v potassium chloride solution and screw the top firmly onto the shaker bottle.
3. Three reagent blank samples prepared by putting 100mL of 6% w/v potassium chloride solution in a shaker bottle. The blanks aims to detect any nitrogen contamination from the extractant and the shaker bottle.
4. Samples and blanks bottles placed on an orbital shaker for 2h.
5. The samples filtered using Whatman filters No 42.
6. Ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) content measured using automated colorimeter.
7. Available NH_4^+-N was calculated using the formula

   (mg kg^{-1} dry compost) = [mean sample conc. - mean sample blank (mg/L)]x[1400/(18 x dry weight of compost in g)]

   Available NO_3^--N was calculated using the formula

   (mg kg^{-1} dry compost) = [mean sample conc. - mean sample blank (mg/L)]x[1400/(62 x dry weight of compost in g)]

   Available NO_2^--N was calculated using the formula

   (mg kg^{-1} dry compost) = [mean sample conc. - mean sample blank (mg/L)]x[1400/(46 x dry weight of compost in g)]

3.1.4.1.4 Available Phosphorus

Weak acetic acid is a widely used extractant for phosphorus. In this method, phosphorus in the samples reacts with added molybdate and ascorbic acid to form a blue complex. Phosphorus concentration is quantified by measuring the absorbance of light by the sample at 660nm.

The sample analysis was conducted in the Crew Laboratory of the School of Geosciences at Edinburgh University. The analysis protocol is given below:

1. Weigh 3g of compost.
2. Add 100mL of 2.5% v/v acetic acid and screw the top firmly onto the shaker bottle.
3. Three reagent blank samples prepared by putting 100mL of 2.5% v/v acetic acid in a shaker bottle. The blank aims to detect any phosphorus contamination from the extractant and the shaker bottle.
4. Samples and blanks bottles placed on an orbital shaker for 2h.
5. The samples filtered using Whatman filters No 42.
6. Phosphorus content measured using automated colorimeter.
7. Available P (mg kg^{-1} dry compost) = [mean sample conc. - mean sample blank (mg/L)]x[100/ (37 x dry weight of compost in g)]

3.1.4.2 Characterization of Silver and Silver doped Titanium Dioxide Nanoparticles.

The NP-concentration in the stock solution was determined using Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). Electric conductivity (EC) and pH measured after dilution using UPW at concentrations of 5, 10, 25 and 50 mg Ag / L.

Zeta Pals instrument from Brookhaven Instruments Corporation was used to determine the particle size and zeta potential of the NP's solution. Solutions with various concentrations of NPs were prepared and analysed using a disposable cuvette. Zeta Pals operations is based on the principles of Dynamic Light Scattering (DLS). DLS is a well-established technique for measuring the size of particles and macromolecules typically in the submicron region down to below 1nm (Malvern 2012). The sample is illuminated by a laser beam and the intensity of the scattered light is measured. The particles in suspension undergo Brownian motion which are caused by thermally induced collisions between the suspended particles and solvent molecules. The analysis of the intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship (Malvern 2012). The method produces an intensity weighted particle size distribution which means that the presence of oversize particles can dominate the particle size result. The diameter measured using DLS is called the hydrodynamic diameter and refers to the way the particle diffuses within a fluid. Diffusion depends on the particle size and surface, and the concentration and type of ions in the medium. This means

that particle size will be larger using DLS method compared to the electron microscopy where the actual size of the particle can be obtained.

Zeta potential measurements are based on phase analysis light scattering. Zeta potential can be used to estimate the stability in aqueous solutions. The electrostatic stabilization increases as the zeta potential increases and becomes very small compared to the present Vander Waals attraction when the zeta potential approaches zero. Instability occurs in aqueous solutions with a zeta potential value close to zero which can result in aggregation, sedimentation and phase separation. The dividing line between stable and unstable dispersions is generally taken as either +30 or −30 mV. Particles with zeta potentials more positive than +30 mV or more negative than −30 mV are normally considered stable. Particles with zeta potential between −30 and +30 mV are considered unstable; these will precipitate sooner or later depending on the density of aggregation of the particles (Shieh, Chen et al. 2012).

The equilibrium achieved in the steady state of a colloidal system is a balance between hard-core repulsions, Van der Waals attractions, screened Coulomb interactions, and hydrodynamic forces. The NPs suspensions are stable for long when there are negative surface charges and prevents two particles from aggregating due to strong columbic repulsion leading to a metastable solution of single NPs. Beyond this metastable state the van der Waals force become dominant and results in the formation of large clusters (Meyer, Le Ru et al. 2006).

Cary 100 Scan UV-visible Spectrophotometer from Agilent technologies was used to measure UV-visible of AgNPs solutions. The particle size of AgNPs dispersions can be analysed with the use of UV absorption spectroscopy. The dispersions of AgNPs display intense colours due to the plasmon resonance absorption. Surface Plasmon Resonance is a collective excitation of the electrons near the surface of the particles. Electrons are limited to specific vibrations modes by the particle size and shape. Therefore, AgNPs have characteristic optical absorption spectrums in the UV-vis region. The particle size can be determined by analysing the optical spectra using Mie theory (Desai, Mankad et al. 2012). The height of the resulting peak is correlated to the particle size while the width of the peak is correlated to the particle size distribution. Absorption at 392 nm and 450 nm corresponds to a particle size of 3.7nm and 60nm respectively (Desai, Mankad et al. 2012). The spherical AgNPS shows only a single SPR band the absorption spectra whereas anisotropic NPs could show two or more SPR bands depending on the shape of NPs (Pal, Tak et al. 2007).

3.1.4.3 Monitoring of Organic matter temporal Evolution using Excitation Emission Matrix Fluorescence Spectroscopy

The production of a 3D plot of fluorescence excitation wavelength, emission wavelength and intensity allows the visualization of a range of fluorophores and their relative positions. Chromophores are defined as the molecules or moieties that absorb light and fluorophores as the fraction of chromophores that re-emit light at longer wavelengths (Mopper, Feng et al. 1996). An additional feature of excitation emission matrix fluorescence spectroscopy (EEM) is the vast array of data available for interpretation within an EEM. Each EEM is a composite of emissions scans from a single sample recorded at incrementing excitation wavelengths and arranged in a grid (excitation x emission x intensity). The wavelength at which absorption (excitation) and emission occur is specific to the molecule (Lacowicz 2006) and depends on the specific chemical structure as well as other factors including sample matrix and temperature. At low concentrations, measured peak intensity is directly proportional to the concentration of the responsible fluorophore in the solution (Henderson, Baker et al. 2009).

The observed, distinct fluorescence properties of HS provide useful diagnostic criteria for distinguishing between FA and HA from the same source as the molecular components of FA and HA differ with source (Senesi, Miano et al. 1991). The groups of fluorophores identifying by fluorescence are commonly named humic-like, fulvic-like and protein-like because their fluorescence occurs in the same area of optical space as standards of these materials. Excitation and emission wavelength boundaries are operationally defined in five regions based on supporting literature (Fig. 3-3): (I) aromatic protein I (API), (II) aromatic protein II (APII), (III) fulvic acids-like (FA), (IV) soluble microbial by product like (SM) and (V) humic acids (HA) (Chen, Westerhoff et al. 2003a). The regions API and APII are related to simple aromatic proteins such as tyrosine. The region SM is related to soluble microbial byproduct-like, material. The region HA is related to humic acid like substances and the region FA is related to fulvic acid like substances. The shift of primary peaks toward higher excitation wavelengths may be indicative of the formation of increasing molecular size components, which is consistent with the formation of humic acid-like substances during composting treatment (Stevenson 1994, Antizar-Ladislao, Lopez-Real et al. 2006).

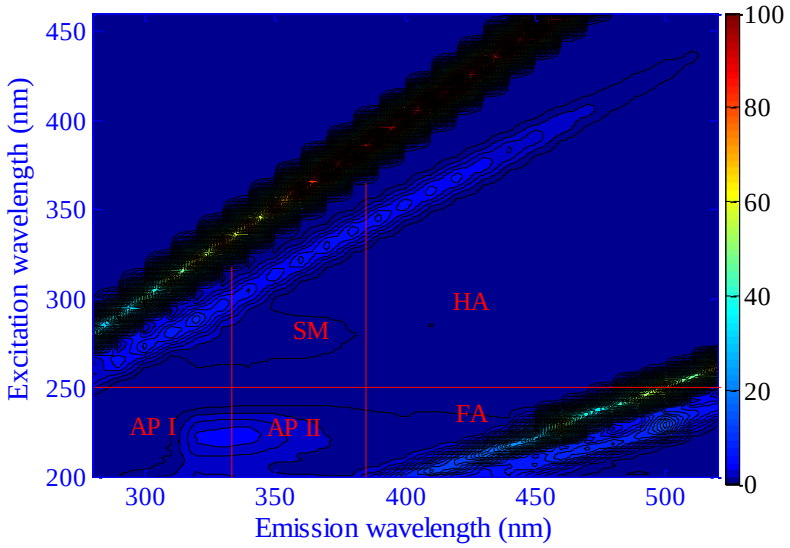

Fig 3-3. Excitation and emission wavelength boundaries definition according to Chen et al. (2003a).

EEM fluorescence spectroscopy technology was used to monitor the formation of humic substances during composting. Contour maps of EEM spectra were obtained on extracts of compost [0.5g (dw) compost to 20ml 0.01M NaOH] after shaking in an orbital shaker for 18h at 75 rpm under N_2 atmosphere in dark and filtration through 0.45 μm filter (Millipore). A Perkin-Elmer LS 55 fluorescence spectrophotometer equipped with FLWinlab for data processing was used for sample analysis. The emission wavelength (Em) range fixed from 200 to 600nm, whereas the excitation (Ex) wavelength increased from 200 to 600 nm keeping a constant $\Delta\lambda$ of 25 nm. The concentrated compost extracts were diluted to eliminate the inner-filter effect (IFE). Compost extracts were fractionated based on solubility of HS at various pH levels to identify the peaks that appeared in EEM spectra. The alkaline compost extract was acidified to pH lower than 2 using 1N HCI (2 mL HCl added to 10 mL extract). The sample allowed standing at room temperature for 24 hours and the insoluble HA aggregates were separated from soluble FA by centrifugation at 5000 x g (RCF). The pellet collected in the bottom of the centrifuge tubes was diluted using 12 mL of 0.01M NaOH and constitutes the HA. The supernatant constitutes the FA fraction. It is called FA fraction because of the possible presence of other compounds (e.g. amino acids, aromatic proteins) apart of FA.

The three-way data (excitation wavelength, emission wavelength and intensity) for each analyzed sample exported to excel files. Contour maps were created using the software Matlab Version 2009a.

The used methodology included the qualification and the quantification of the HS. The qualification was conducted by diluting the samples to have a value of 0.3Å when measure the UV absorbance at 254 nm. By diluting using this method, the EEM matrix provides information of the substances present in the analyzed sample. The peaks of the substance with the higher concentration will be dominant in the matrix.

The quantification of HS was conducted in mature samples following fractionation using the method described above. The concentration of HA and FA was determined using the concentration method of fluorescence spectroscopy. The calibration curve for HA was obtained using the Leonardite HA standard supplied from IHSS. A 500 mg/L stock solution of Leonardite HA standard was prepared by diluting 0.025 mg of the standard in 50mL of 0.1 N NaOH, stirring for an hour at room temperature and filtering through 0.45 μm filter. No residuals observed on the filter. The calibration curve for FA was obtained using the Pahokee Peat FA standard supplied from IHSS. A 500 mg/L stock solution of Pahokee Peat FA standard was prepared by diluting 0.025 mg of the standard in 50mL of UPW, stirring for an hour at room temperature and filtering through 0.45 μm filter. No residuals were observed on the filter. The calibration curves are given in Appendix A.

3.1.4.4 Quality Assurance and Quality Control

Quality assurance (QA) is the definitive program for laboratory operation that specifies the measures required to produce defensible data of known precision and accuracy (ASTM 2005). Adequate training and following of standard operation procedures (SOPs) are essential to obtain reliable results.

The quality control is including in analytical method or SOPs for each analysis. Quality control elements applicable to the laboratory experiments of this study include:

- Control experiments to compare the results with the results obtained from the control.

- Analyze at least one blank sample with each sample set, after the daily calibration standard or after highly contaminated samples if carryover is suspected.
- Determination of the method detection limit. Results below the method detection limit are reported as "not detected". The detection limit of fluorescence spectroscopy for HA and FA was estimated using the standard reference materials and detected to be 1 mg/L (see Appendix A 1.4).
- Calibration. The calibration standards shall not have more than one order of magnitude between concentrations. A calibration standard shall be used to check the instrument performance where no daily calibration is required according to the manufacturer's recommendations. The standard reference materials (Leonardite HA standard, Pahokee Peat HA standard and Pahokee Peat FA standard) were purchased from IHSS.

Sometimes one or more results differ greatly from the other values. Theoretically, no result should be rejected, because it may indicate either a faulty technique that casts doubt on all results or the presence of a true variant in the distribution (ASTM 2005).

3.1.5 Results and Discussion

3.1.5.1 NPs characterization

3.1.5.1.1 Ag-TiO_2NPs

Commercial water-based NPs solution was supplied and used for the experiments as received. The colour of the original stock solution was bluish white/yellow (Fig 3-4). Silver and titanium concentrations of the original solution were determined using ICP-MS: 100 mg/L Ag and 4500 mg/L Ti.

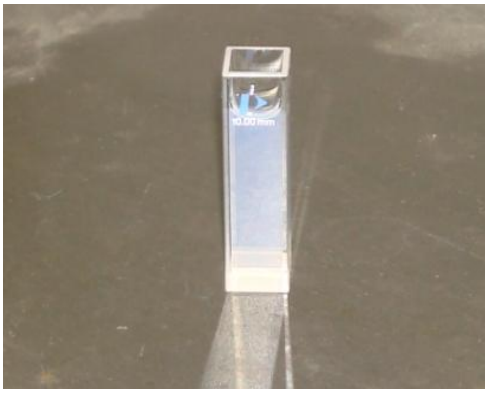

Fig. 3-4. Ag-TiO_2NPs solution with concentration 50 mg/L AgNPs and 2250 mg/L TiO_2NPs.

EC, pH, zeta potential and particle size were measured at different concentrations: 5, 10, 25 and 50 mg Ag /L (Table 3-4). It was observed that pH values were lower for more diluted solutions while the EC values were higher for more concentrated solutions. A linear correlation between EC and AgNPs was evident (R^2=0.9999). Recorded zeta potential values ranged between -37.3 to -51,79 mV which indicated that the solutions remained stable at all concentration levels. The particle size increased for more diluted solutions with an effective diameter of 24.2 and 30 nm for the solution with concentration of 50 and 5 mg Ag /L respectively. This may indicate that agglomeration occurs in UPW at low concentration values.

Table 3-4. Particle size, pH, EC and zeta potential for AgTiO_2NPs solution at different concentrations.

Sample	**pH**	**EC (µS/cm)**	**Zeta Potential (mV)**		**Particle Size (nm)**	
			Av	St Error	Mean	St Error
5 mg/L	7.03	35	-37.3	0.61	30	0.1
10 mg/L	7.34	70	-42.94	0.74	28.1	0.1
25 mg/L	7.52	171	-51.79	0.84	26	0.1
50 mg/L	7.56	339	-47.54	1.25	24.2	0.1

The particle size distribution for a solution with concentration of 50 mg Ag/L is presented in Fig 3-5. The effective diameter of the sample was 24.2 nm and the polydispersity was 0.281. Effective diameter represents an average size of the particles in the sample and polydispersity is a measure of the non-uniformity that exists in the particle size distribution. Polydispersity values are close to zero (0.000 to 0.020) for monodisperse solutions or nearly monodisperse samples, small (0.020-0.080) for narrow distributions and larger for broader distributions. G(d) is the relative percentage contribution of the size range. The largest group has the frequency of 100 (BIC 1999). Two main peaks appear in the graph. The first peak is around 13 nm and

the second peak in around 60 nm (Fig. 3-5). According to the manufacturer the agglomeration index is 2-4 and the primary particle size is 8 nm. Taking into account the error of the DLS method due to the determination of the hydrodynamic diameter, the resulted agglomeration seems to be in accordance with the product specifications. The UV-Vis analysis of Ag-TiO_2NPs solutions didn't result in any peaks. This can be attributed to the presence of TiO_2NPs at high concentrations in which is attributed the white colour of the solution.

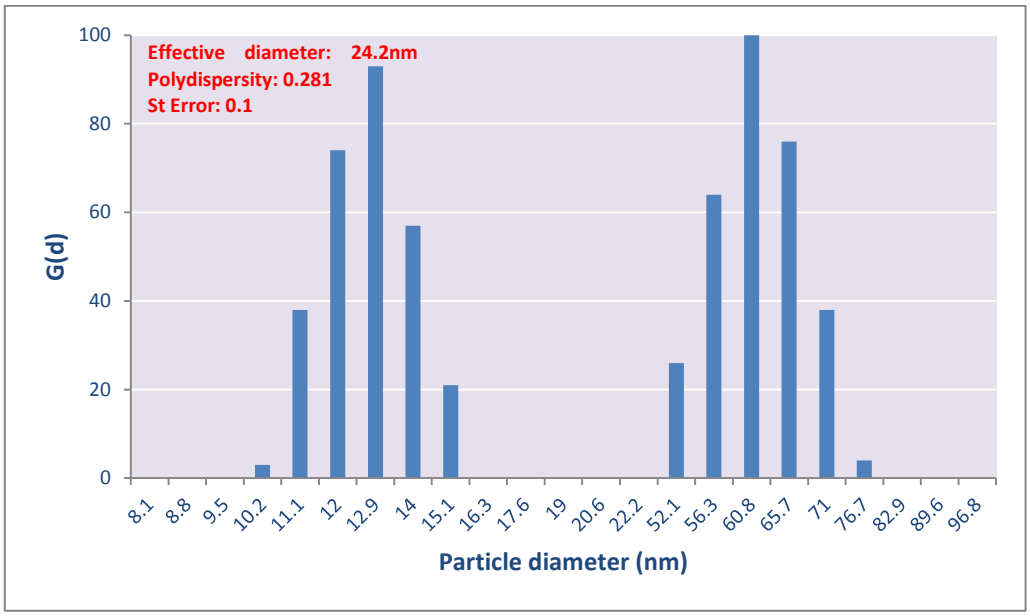

Fig 3-5. Particle size distribution for silver doped titanium dioxide (TiO_2) nanoparticles solution at a concentration of 50 mg Ag / L.

3.1.5.1.2 AgNPs

The colour of AgNPs stock solution was orange-brown. A solution with concentration 50 mg Ag /L is presented in Fig. 3-6. EC, pH and particle size were measured at different concentrations: 5, 10, 25, 50 mg Ag /L and matrix solution (Table 3-5). The matrix solution was diluted using the same dilution factor as the one used to prepare the 50 mg Ag / L solution. Zeta potential measurements were conducted however the results exhibit high standard deviations so it was decided to discard the results as reliable reading could not be achieved. Zeta potential can be affected by changes in pH, conductivity (salt concentration) or changes in the concentration of an additive (e.g ionic surfactant, polymer). The pH was slightly alkaline and the conductivity values were low, so it seems unlikely that the reading was affected by either pH or EC

values. The matrix should have be used as a control to check if the unreliable readings could be attributed to the presence of stabilizers such as polysorbate.

Table 3-5. Particle size, pH and EC for AgNPs solutions with different concentrations and for the matrix of the solution.

Sample	pH	EC (µS/cm)	Particle Size (nm)	
			Av	St Error
5 mg/L	4.30	6	45.0	0.5
10 mg/L	4.71	13	41.8	0.3
25 mg/L	5.38	41	39.6	0.3
50 mg/L	5.82	71	38.3	0.1
Matrix	6.13	115	9.7	0.1

Fig 3-6. AgNPs solution with concentration 50 mg Ag / L.

The particle size distribution for a solution with concentration of 50 mg Ag/L is presented in Fig 3-7. The effective diameter of the sample was 42.1 nm and the polydispersity was 0.286. Two main peaks appeared in Fig. 3-7. The first peak was around 20 nm and the second peak was around 80 nm. According to the manufacturer the primary particle size is 15 nm. Taking into account the error of the DLS method due to the determination of the hydrodynamic diameter, the first peak seems to satisfy the product specifications. The second peak, which is dominant in the solution, may indicate agglomeration of AgNPs. The matrix solution suggested a particle size of around 10 nm (see Appendix C).

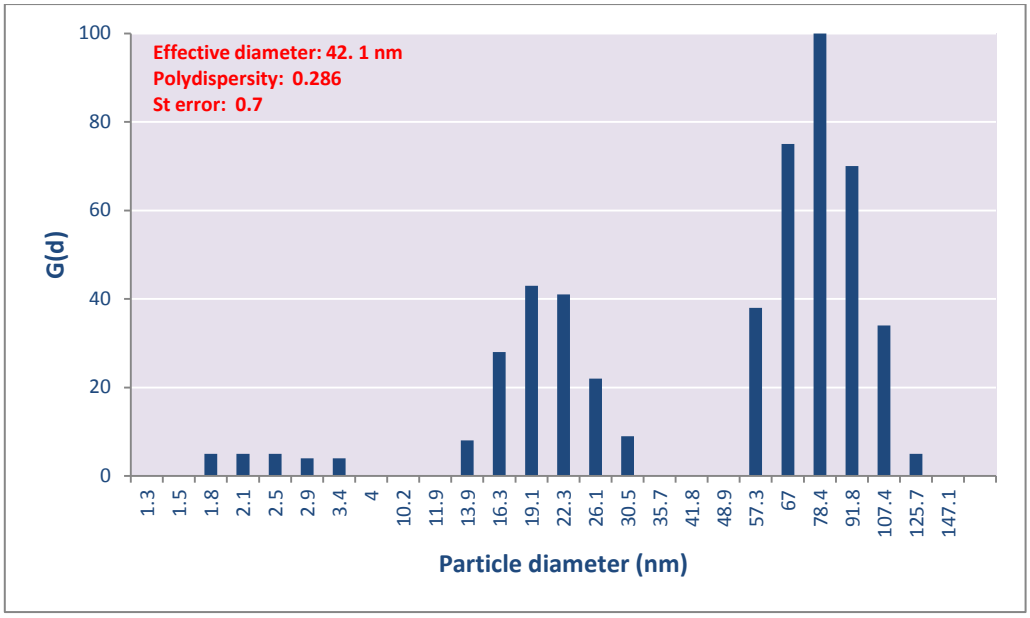

Fig 3-7. Particle size distribution for AgNP's solution at concentration of 50 mg Ag / L.

The results obtained from UV absorbance are presented in Figures 3-8 and 3-9 for concentrations of 5 and 25 mg Ag / L respectively. Both solutions show a peak at 414 nm with different intensity which corresponds to a particle size *ca.* 15 nm.

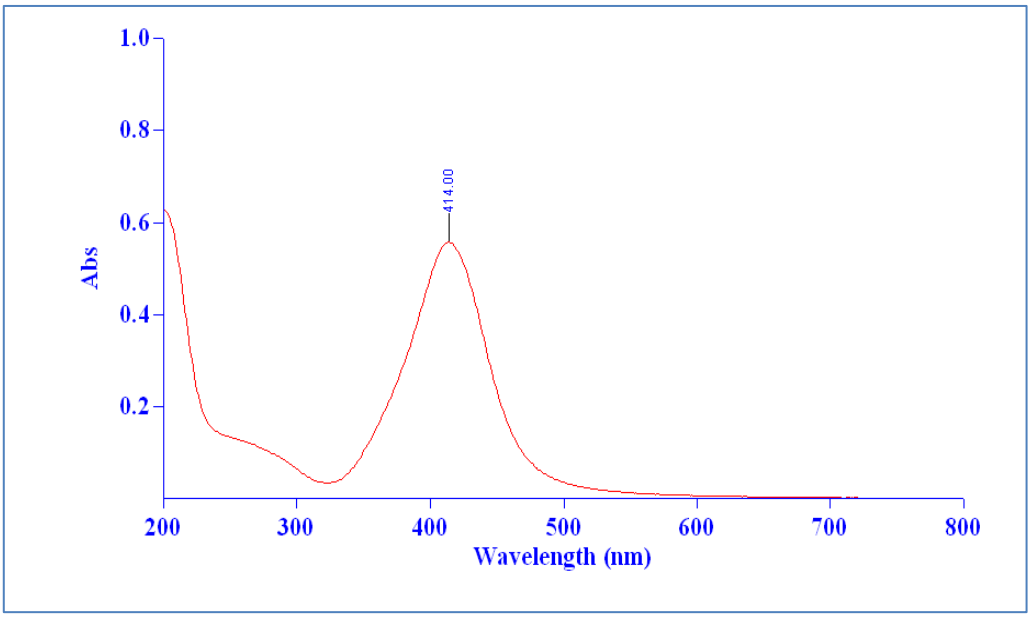

Fig 3-8. UV absorbance of AgNPs solution at concentration of 5 mg Ag / L.

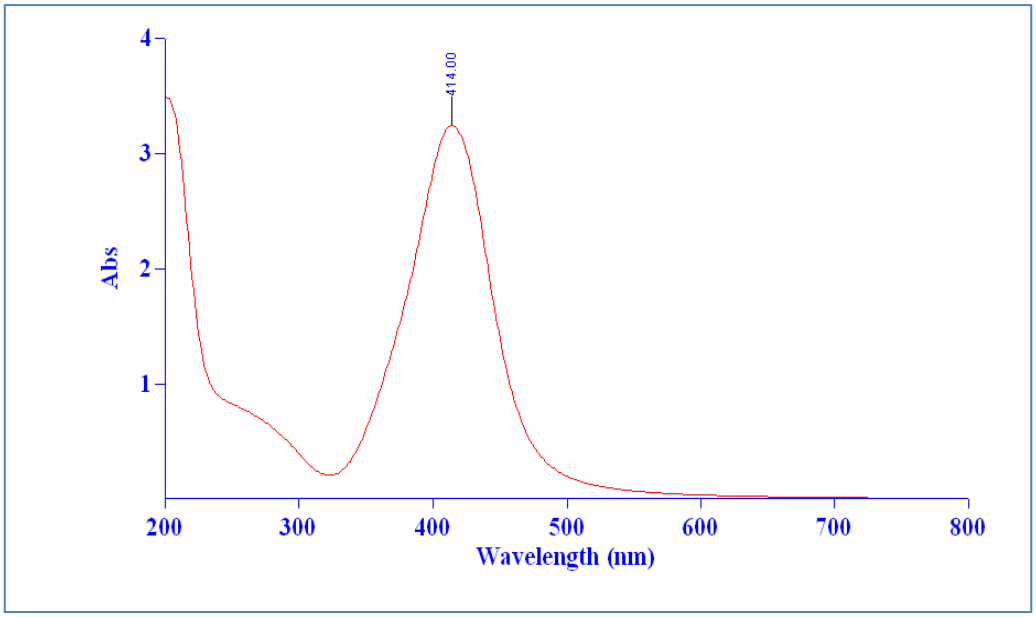

Fig 3-9. UV absorbance of AgNPs solution at concentration of 25 mg Ag / L.

The different methods used to determine the particle size of the NPs used for the purpose of this study provided results which differed substantially.

While UV method resulted in a particle size *ca.* 15 nm without any differences in particle size between solutions of 5 and 25 mg Ag/L the DLS method resulted in an average particle size of *ca.* 38 nm with two different peaks one at *ca.* 20 nm and one at *ca.* 80 nm. The DLS method produces an intensity weighted particle size distribution which means that the presence of oversize particles can dominate the particle size result (Malvern 2012). The diameter measured using DLS technique measures the hydrodynamic diameter which means that particle size will be larger using DLS method compared to other techniques such as electron microscopy where the actual size of the particle can be obtained.

3.1.5.2 Biodegradable MSW characterization.

Biodegradable MSW was analysed before composting (Day 0) to determine pH, ash content, C/N ratio and OM. The pH was 5.80 ± 0.12 which indicate that the waste was slightly acidic. The ash content was 5.21 ± 0.22 % and the C/N ratio was 43.49 ± 2.90. OM was monitored using EEM fluorescence spectroscopy (See EEM spectra in Fig 3-10). In order to avoid possible inner filter effect, samples were diluted to have absorbance values lower than 0.05Å at λ=254nm.

Three main peaks appeared at Ex/Em of 220/310, 280/310 and 320/440 nm/nm. The peaks at Ex/Em 220/310 and 280/310 nm/nm are characteristic for aromatic proteins

(API) and soluble microbial by product (SM) respectively. The peak at Ex/Em 320/440 nm/nm can be attributed to terrestrial FA like because this peak is characteristic for fulvic acids.

Ag-TiO_2NPs and AgNPs were added to biodegradable MSW at different concentrations and OM extracts were analysed. Separated spectra were obtained in order to investigate if there is any effect in EEM spectra (peaks location and intensity) due to the presence of NPs. There were no differences observed at the location and intensity of the peaks in EEM spectra compared to the EEM spectra presented in Fig 3-10. This result was expected as Ag and TiO_2 do not exhibit any fluorescence properties.

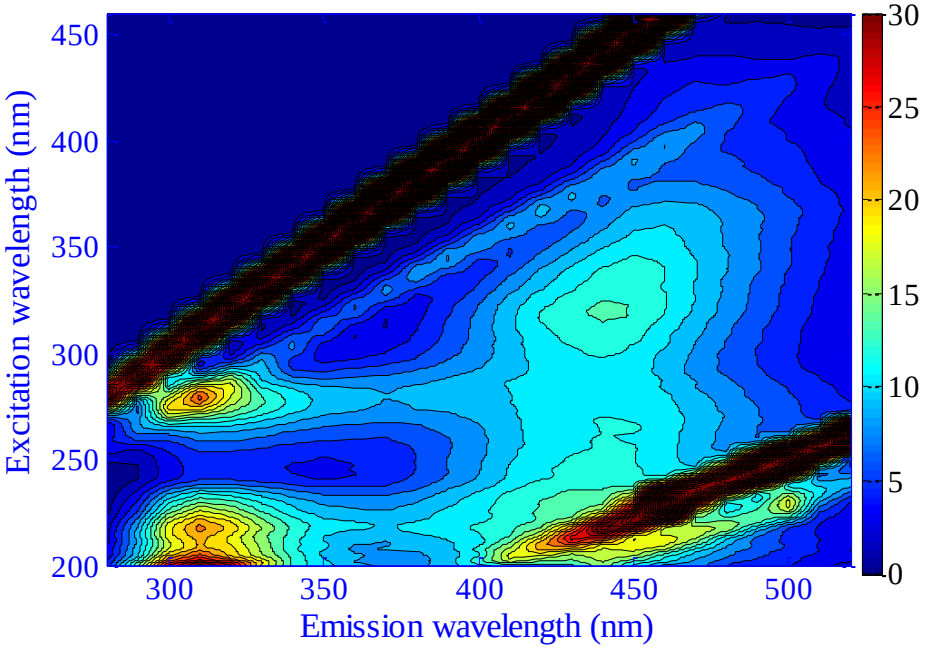

Fig 3-10. EEM spectra for biodegradable MSW.

3.1.5.3 Microbial activity inhibition using NaN_3

The initial pH of composting mixture was 5.91, then increased slightly to 6.04 at day 4 and reduced to 4.64 at day 21 (Fig. 3-11). This may indicate the inhibition of nitrifying bacteria growth during composting due to the presence of NaN_3. Also, no fungi growth was observed during the 21 days of NaN_3-inhibited composting. This may imply that NaN_3 inhibited also the growth of fungi.

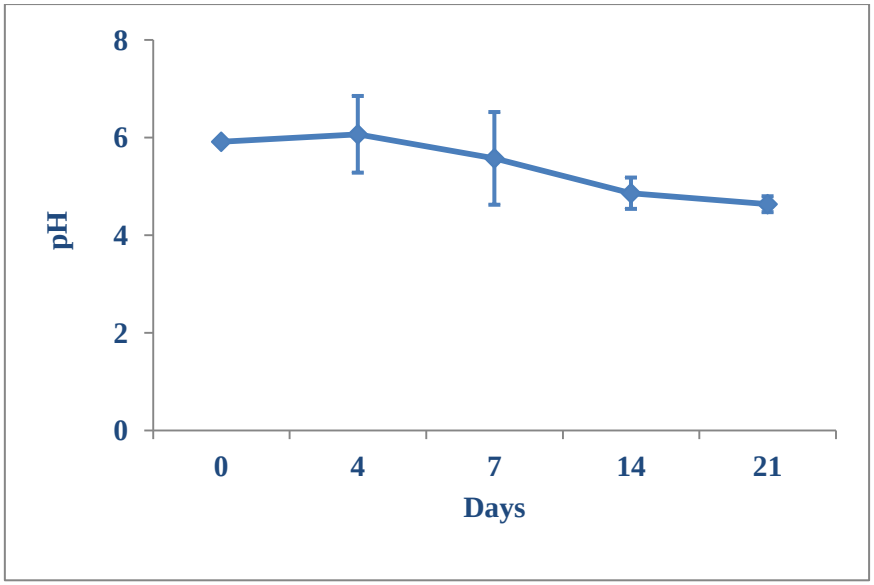

Fig 3-11. The pH values during NaN_3-inhibited composting.

The MC values were between 59 to 66% during composting (Fig. 3-12). The MC was determined at 45°C for 72 h because of the presence of NaN_3 due to the risk of explosion when this substance is exposed to high temperatures (Sigma-Aldrich 2012). The reliability of the method was checked by comparing the MC values of the compost samples at days 4, 7, 14 and 21 that resulted when drying the samples at 105°C for 24 h and 45°C for 72 h. The MC of the samples dried at 45°C for 72 h had the same MC value as the MC value of the samples dried at 105°C for 24 h.

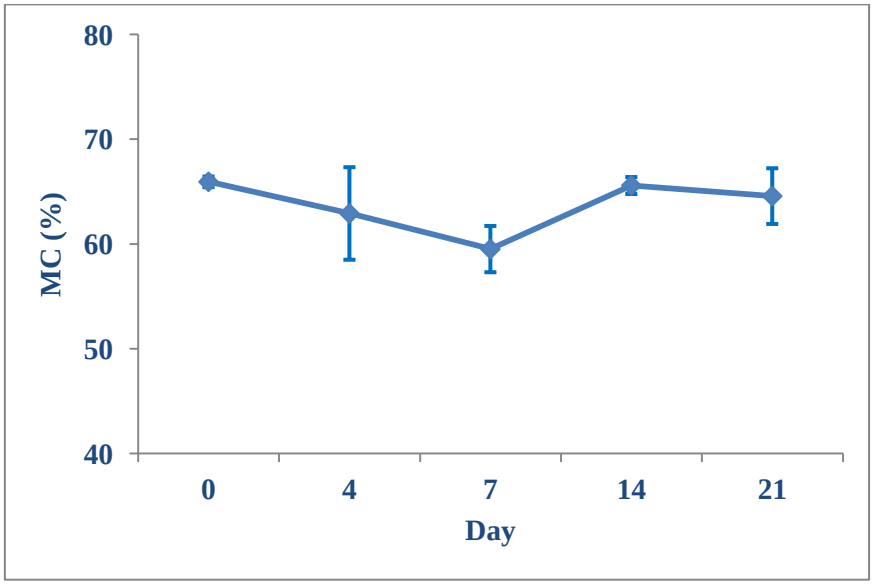

Fig 3-12. MC values during NaN_3-inhibited composting

The percentage of loss weight after 21 days of NaN_3-inhibited composting was as low as 2.88 (Table 3-6). This may be attributed both to the abiotic losses and the possible decomposition occurred.

Table 3-6. Dry weight and % weight loss of waste after 21 days of NaN_3-inhibited composting.

	Dry weight (g)		(%) Weight Loss	
	Av	**StDev**	**Av**	**StDev**
SA	26.46	0.29	2.88	1.06

Contour maps of EEM spectra were obtained on extracts of compost [1g (w/w) compost to 50ml UPW] after shaking in an orbital shaker for 2h at 75 rpm under N_2 atmosphere in dark and filtration through 0.45 μm filter (Millipore). The samples were diluted to have UV absorbance of 0.05Å at λ=254 nm. The EEM contour maps for days 4,7,14 and 21 are presented in Fig 3-13.

At day 4, four main peaks appeared in EEM spectra, at Ex/Em 220/320, 220/360, 280/320 and 280/360 nm/nm. At day 7 no major differences appeared in peaks location in EEM spectra compared to the peaks appeared in EEM spectra at day 4. This may indicate that the first seven days the presence of NaN_3 inhibited effectively the microbial activity.

At day 14, three main peaks appeared at Ex/Em 220/360, 280/320 and 280/360 nm/nm and one shoulder at Ex/Em 250/440 nm/nm. The absence of the aromatic protein's peak at Ex/Em 220/320 nm/nm does not imply that there is no quantity of the aromatic protein identified with this peak. This may imply that the proteins identified using this peak are not any more dominant in the analysed compost extract.

It is clear that there is a trend for aromatic proteins identified with the peak at Ex/Em 220/320 nm/nm to combine together resulted in aromatic protein identified at Ex/Em 220/360 nm/nm. This may explain why the peak at Ex/Em 220/360 nm/nm is broader compared to the peaks appeared in EEM spectra at day 4 and day 7 and has higher intensity. Similarly, there is a trend for aromatic proteins identified with the peak at Ex/Em 280/320 nm/nm to combine together resulted in aromatic protein identified with the peak at Ex/Em 280/360 nm/nm. This may explain why the peak at Ex/Em 280/360 nm/nm is broader compared to peaks appeared in EEM spectra at day 4 and day 7 and has higher intensity. It is suggested that the appearance of a shoulder at

Ex/Em 250/440 nm/nm may have resulted from the combination of aromatic proteins identified of the peak at Ex/Em 220/360 nm/nm and the aromatic proteins identified with the peak at Ex/Em 280/360 nm/nm. The results indicated that the microbial activity activated the 2nd week of composting resulted in a low degree of decomposition of OM.

At day 21, two main peaks appeared at Ex/Em at 220/360 and 280/360 nm/nm and one shoulder at Ex/Em 250/440 nm/nm. Both of the peaks are broader and have much higher intensity compared to the peaks appeared in EEM spectra at day 14. As above, the absence of the peak at Ex/Em 280/320 nm/nm can be explained due to a shift toward peaks at Ex/Em 280/360 nm/nm. It is clear that there is a trend for aromatic proteins identified with the peak at Ex/Em 280/320 nm/nm to combine together resulted in aromatic protein identified with the peak at Ex/Em 280/360 nm/nm. The shoulder at Ex/Em of 250/440 nm/nm is broader compared to the one at day 14.

The fact that no characteristic peaks of HA and FA appeared at day 21 may indicate that the rate of the decomposition observed is very slow and the time is not enough for the formation of HS. This is in congruence with the weight loss results which are as low as 2.88% following 21 days of composting.

The conclusion is that the presence of 2% NaN_3 in biodegradable MSW inhibited effectively the microbial activity the 1st week of composting. The microbial population activated the 2nd week of composting but the decomposition rate was so low that did not result in the formation of HS following 21 days of composting.

The results are very interesting and may explain the evolution of the decomposition process. It seems that aromatic proteins identified with the peak at Ex/Em 280/320 nm/nm combine together resulted in aromatic protein identified with peak at Ex/Em 280/360 nm/nm. Also, aromatic proteins identified with the peak at Ex/Em 220/320 nm/nm combine together resulted in aromatic protein identified with the peak at Ex/Em 220/360 nm/nm. The combination of aromatic protein identified with the peak at Ex/Em 220/360 nm/nm results in a peak at Ex/Em 250-260/440-450 nm/nm which is identified as one of the peaks characterized the FA. The combination of aromatic protein identified with the peak at Ex/Em 280/360 nm/nm results in a peak at Ex/Em 300-320/420-440 nm/nm which is identified as another of the peaks characteristic for FA.

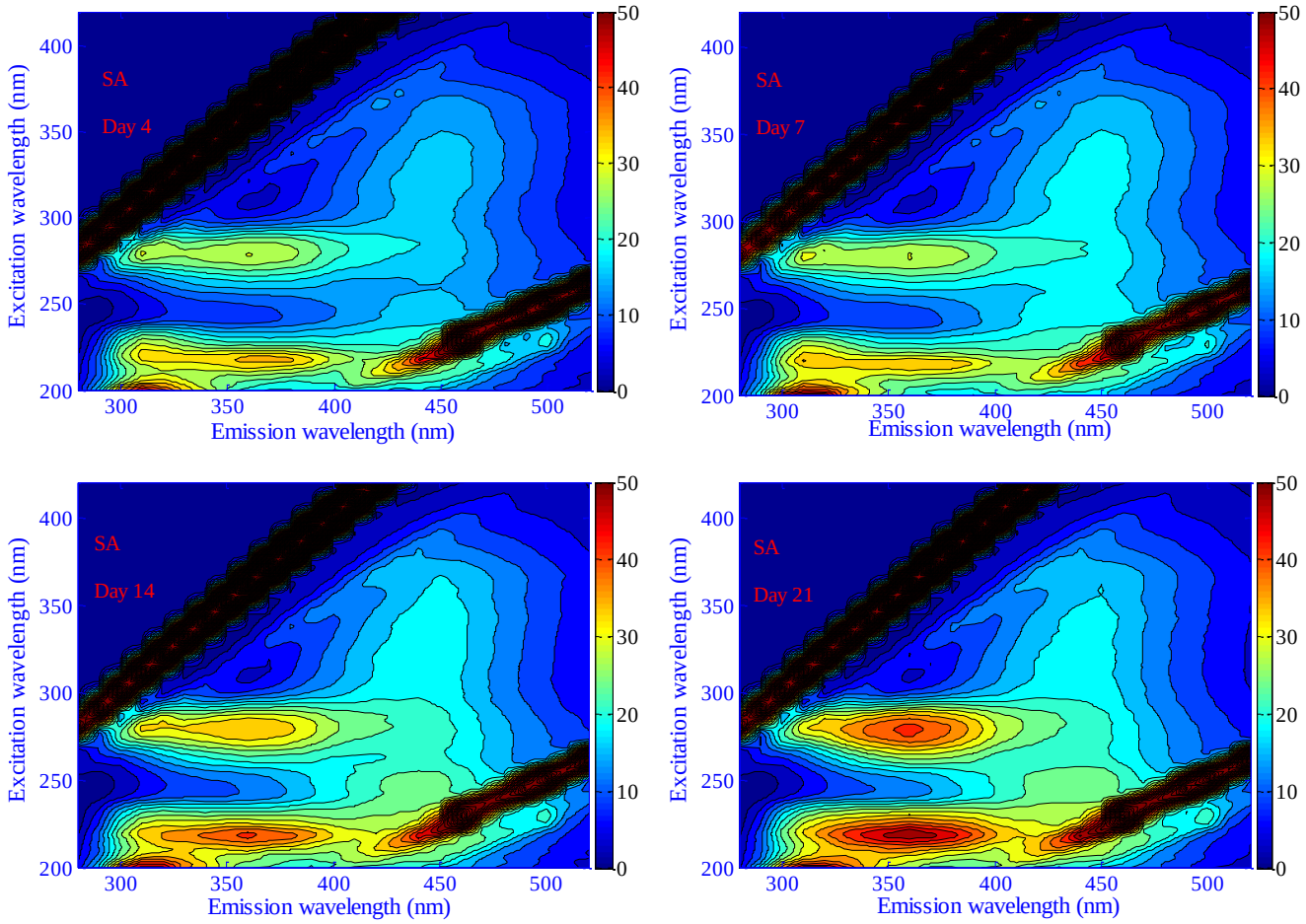

Fig 3-13. EEM contour maps for days 4, 7, 14 and 21 of NaN_3 inhibited composting experiment.

3.1.5.4 Organic waste composting contaminated with silver doped titanium dioxide nanoparticles

The compost pH values for the different experiments: AT 0; AT 5; AT 10; AT 20 and AT 50 (see sub-chapter 3.1.3.2 for explanation) are given in Figure 3-14. The initial pH of composting mixtures was acidic (5.73-5.91) and then increased to alkaline (7.52-7.84) at day 4. At day 7, the pH values were at the same levels as were at day 4 for AT 5, AT 10 and AT 20 whereas for AT 0 and AT 50 the pH increased to 8.26 and 8.80 respectively. At days 14 and 21 the pH values slightly decreased for AT 5, AT 10 and AT 20 and were between 7.23-7.48 whereas for AT 0 and AT 50 the values were between 8.28-8.37.

The increase of pH from acidic to alkaline at the initial stage of composting, reflects the loss of organic acids through volatilization and mineral decomposition and the

release of ammonia through mineralization of organic nitrogen (Finstein and Morris 1975).

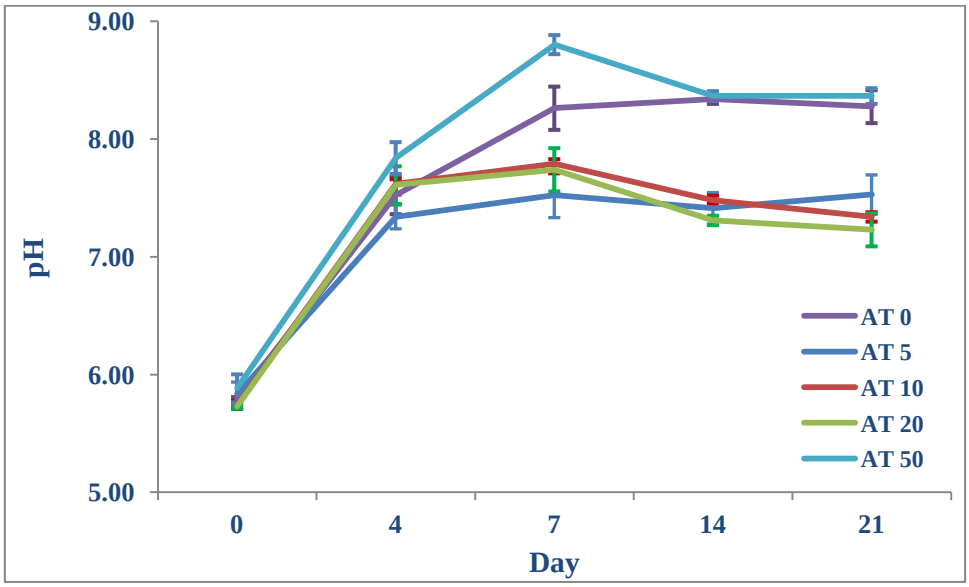

Fig 3-14. The pH values of Ag-TiO_2-NP-contaminated compost during composting.

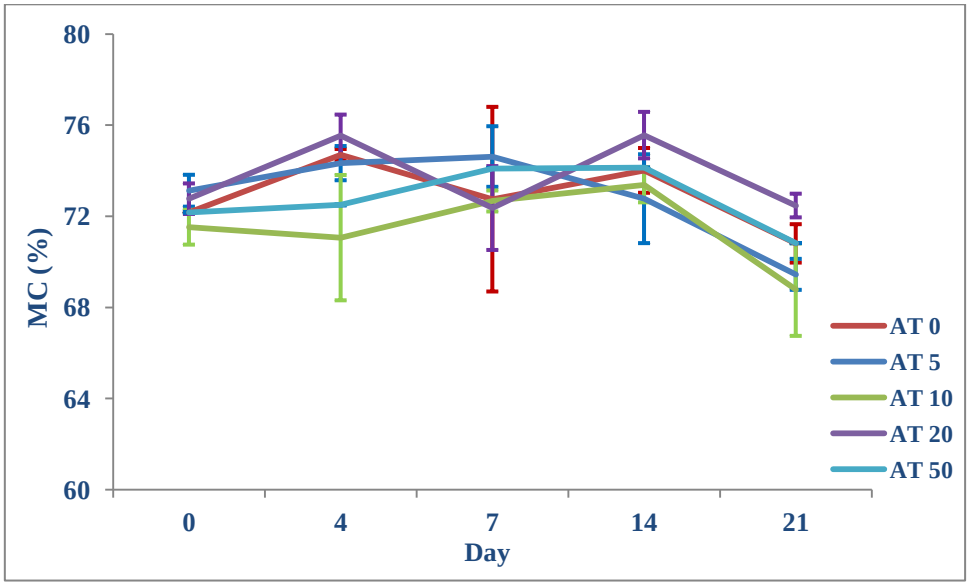

Fig 3-15. MC values for the different experiments conducted using Ag-TiO_2-NPs.

The MC values are given in Figure 3-15. The MC values were kept at the same levels for all the experiments and were between 68.78-75.56%. Keeping MC constant during composting is essential to compare different treatments because different MC levels can result in different decomposition levels (Antizar-Ladislao, Lopez-Real et al. 2006).

The ash content values are given in Figure 3-16. The initial ash content was between 5.18-5.46% for all the experiments. At day 4, the ash content increased to 6.25-6.36% for all the experiments except of AT 50 where the ash content was clearly higher (6.75%). At day 7, the ash content value for AT 50 was distinctly higher. The ash content of AT 50, at day 21, was 9.24% with very low standard deviation and distinctively higher compared with AT 0, AT 5 and AT 20 where the ash content values were between 8.06-8.30% and AT 10 which was 7.23 much lower from all the other experiments. The ash content represents mainly the inorganic carbon of the samples. The difference between the ash content value and the initial ash content can be assumed that is the inorganic carbon produced during composting. It is clear that the 1st week of composting there was much higher production of inorganic carbon compared to the 2nd and 3rd week.

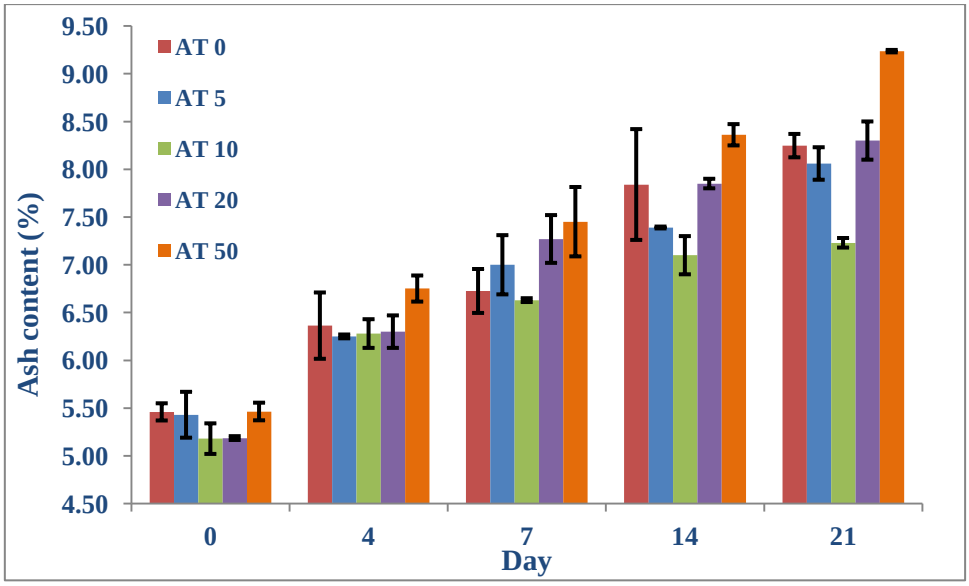

Fig 3-16. Ash content percentage for the different experiments conducted using Ag-TiO_2-NPs.

The compost weight on a dry basis and the percentage of the weight loss following 21 days of composting are given in Table 3-7 and Figure 3-17.

Table 3-7. Weight on a dry basis and percentage of weight loss of waste after 21 days of composting.

	Dry weigth (g)		(%) Loss	
	Av	**StDev**	**Av**	**StDev**
AT 0	16.44	0.22	39.68	0.81
AT 5	16.54	0.45	34.88	1.76
AT 10	17.32	0.39	38.69	1.38
AT 20	15.52	0.22	43.05	0.8
AT 50	16.22	0.02	40.47	0.06

The weight loss values were similar for the experiment AT 0, AT 10 and AT 50. The experiment AT 20 showed higher weight loss values whereas the experiment AT 5 showed lower weight loss values but with relative high standard deviation compared with the others.

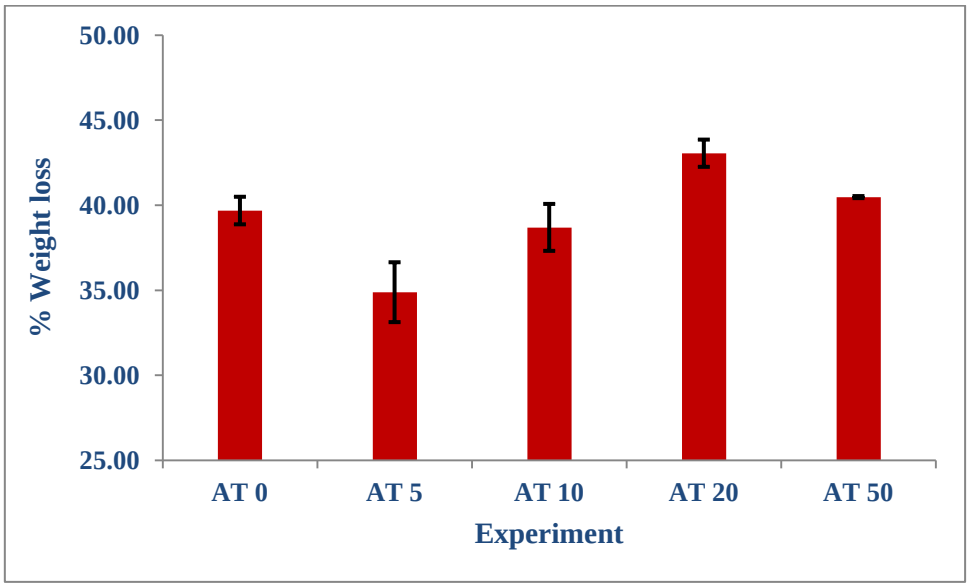

Fig 3-17. Percentage of weight loss of waste after 21 days of composting.

A qualitative analysis (see sub-chapter 3.1.4.3) using EEM fluorescence spectroscopy was performed to monitor the formation of HS.

The EEM spectra, at day 4, obtained for the different treatments are presented in Figure 3-18. At day 4, AT 0 seems to have the lower and AT 10, AT 20 the higher decomposition. AT 0 seems to have the lower decomposition rate because of the higher intensity of the peaks located at Ex/Em 220/310 and 280/310 nm/nm and the broad peak which appeared at Ex/Em 220/380-420 nm/nm and it is absent to the other contour maps. AT 10 and AT 20 show the higher decomposition rate because of the higher peak intensities occurred at Ex/Em 270-360/400-500 nm/nm.

The EEM spectra, at day 7, obtained for different treatments are presented in Figure 3-19. The HS are in the early stages of their formation with FA and HA peaks to be very close. AT 0 and AT 50 have the lower decomposition because the resulted peaks are wider and cover emission wavelengths less than 400 nm while the resulted peaks for AT 5, AT10 and AT 20 are narrower and have shifted to emission wavelengths higher than 400 nm.

The EEM spectra, at day 14, obtained for different treatments are presented in Figure 3-20. AT 0 and AT 50 exhibited the lower decomposition rate because the peaks are wider and cover lower wavelengths. AT 20 exhibited the higher decomposition rate as the resulted peaks have shifted towards higher wavelengths (450 nm).

The EEM spectra, at day 21 and mature samples, obtained for different treatments are presented in Figures 3-21 and 3-22 respectively. AT 20 exhibited the higher decomposition as the resulted peaks have shifted and cover the emission wavelength of 450 nm and the shape is narrower compared to the other treatments. AT 10 exhibited the lower decomposition rate as the resulted peaks are in lower emission wavelengths compared to the other treatments.

The FA fraction of mature samples separated from HA (see sub-chapter 3.1.4.3) and different EEM spectra obtained. The EEM spectra for the FA fraction of mature samples are presented in Figure 3-23. AT 0, AT 20 and AT 50 exhibited the higher peak intensities for the FA fraction. AT 20 resulted peaks for FA were at higher emission wavelengths compared to the other treatments. All the spectra exhibited peaks in the API and SM region at Ex/Em 220/310 and 280/310. Those peaks represent the remaining OM available for degradation. The FA concentrations are given in Table 3-8 (See also Appendix A 1.6).

Table 3-8. The concentration of FA in mg/g for the different treatments.

Sample	**AT 0**	**AT 5**	**AT 10**	**AT 20**	**AT 50**
	mg/g	**mg/g**	**mg/g**	**mg/g**	**mg/g**
1	11.50	8.28	9.83	13.11	10.94
2	10.09	8.74	9.78	13.84	11.61
3	11.88	8.16	9.18	14.00	9.72
Av	**11.16**	**8.39**	**9.60**	**13.65**	**10.75**
St Dev	0.94	0.31	0.36	0.47	0.96

The EEM spectra for the HA of mature samples are presented in Figure 3-24. AT 20 exhibited the higher intensity for HA peaks while the other treatments exhibited similar peak intensities. The HA concentrations are given in Table 3-9 (See also Appendix A 1.2) .

Table 3-9. The concentration of HA in mg/g for the different treatments.

Sample	**AT 0**	**AT 5**	**AT 10**	**AT 20**	**AT 50**
	mg/g	**mg/g**	**mg/g**	**mg/g**	**mg/g**
1	11.22	11.43	11.94	13.19	10.62
2	10.54	10.43	10.75	11.86	10.83
3	11.07	8.95	10.14	12.01	10.34
Av	**10.95**	**10.27**	**10.94**	**12.36**	**10.59**
St Dev	0.36	1.25	0.92	0.73	0.25

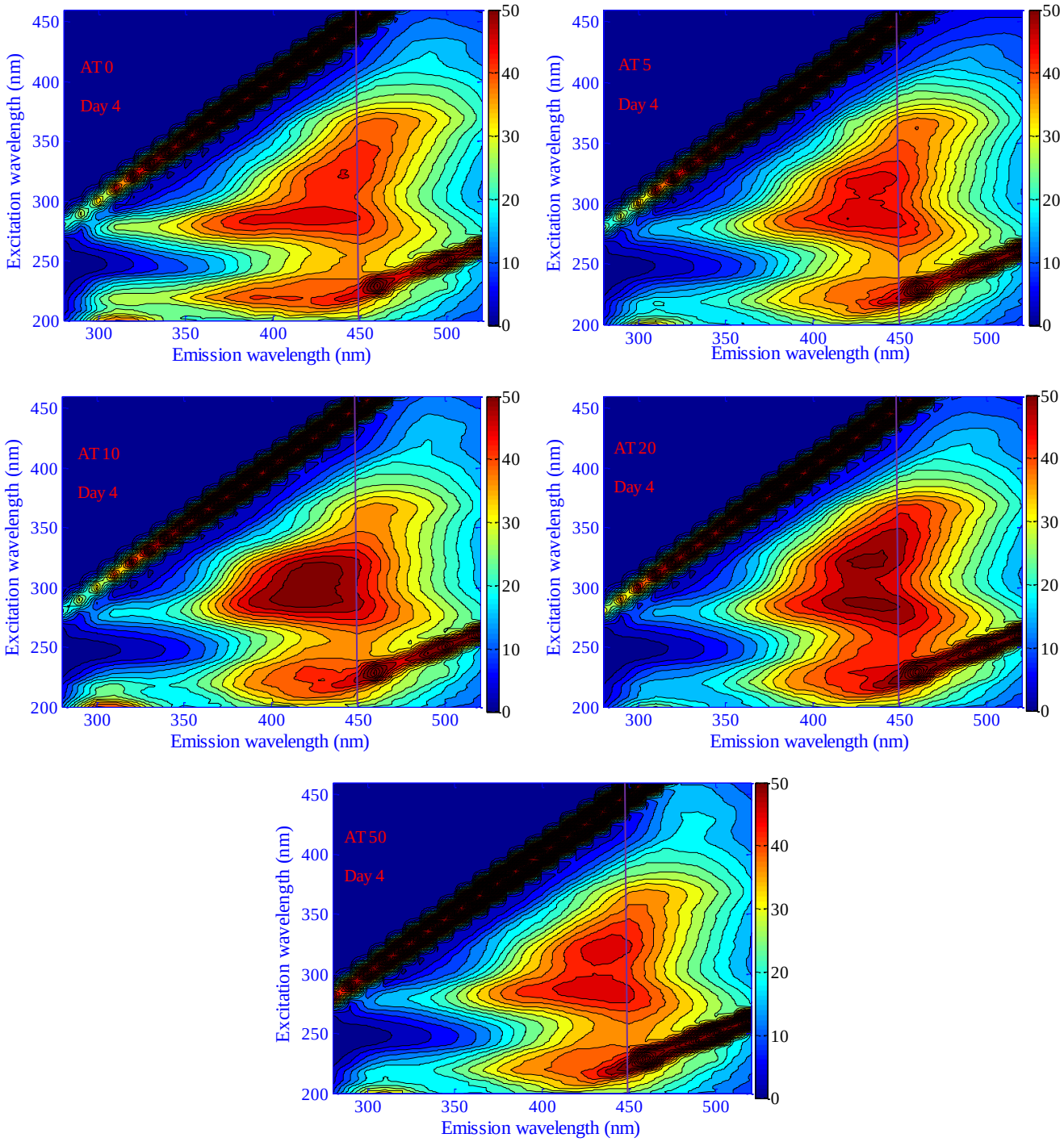

Fig 3-18. EEM contour maps of day 4 for the different experiments (AT 0 through AT 50) conducted using Ag-TiO_2-NPs.

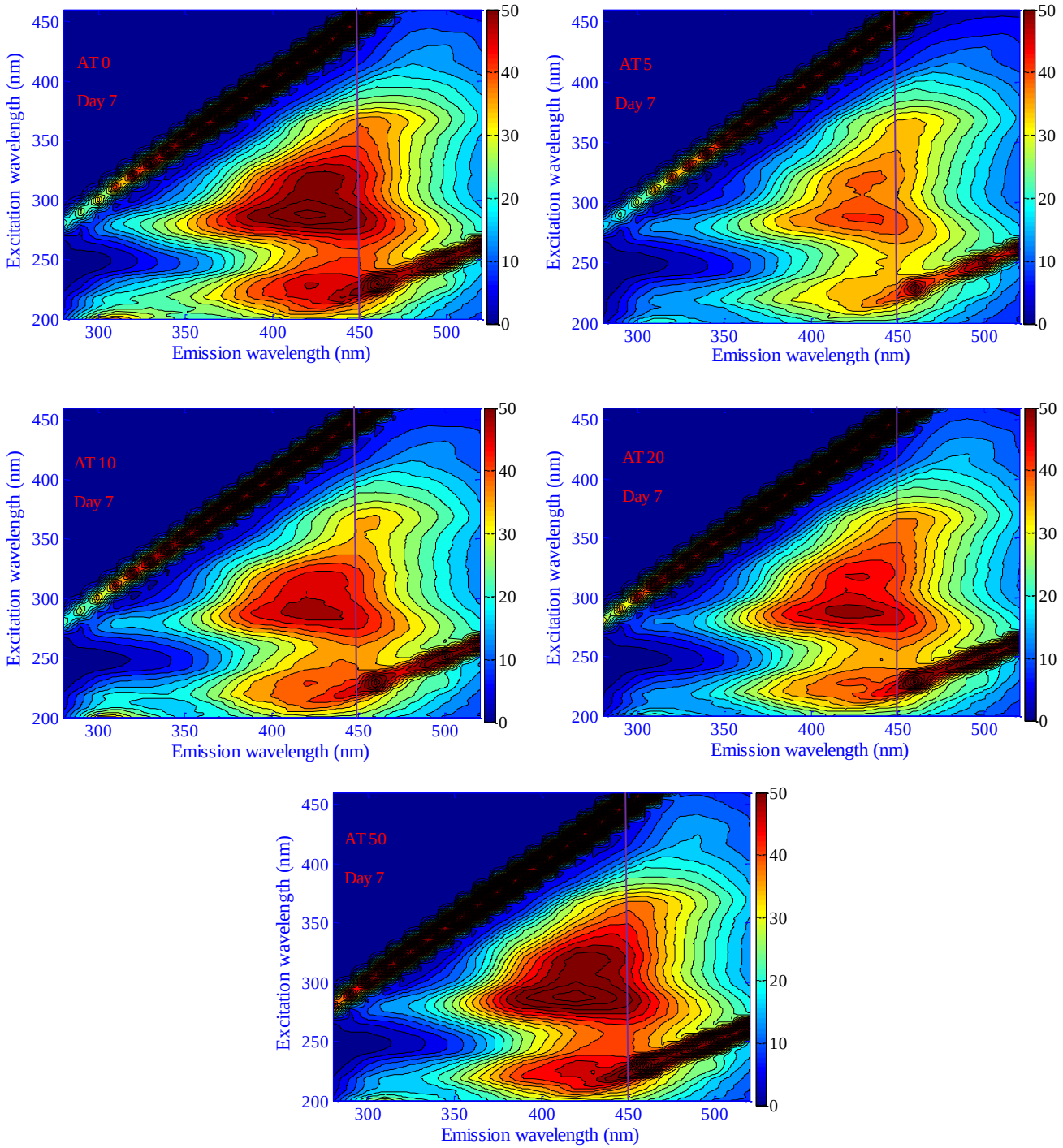

Fig 3-19. EEM contour maps of day 7 for the different experiments (AT 0 through AT 50) conducted using Ag-TiO_2-NPs.

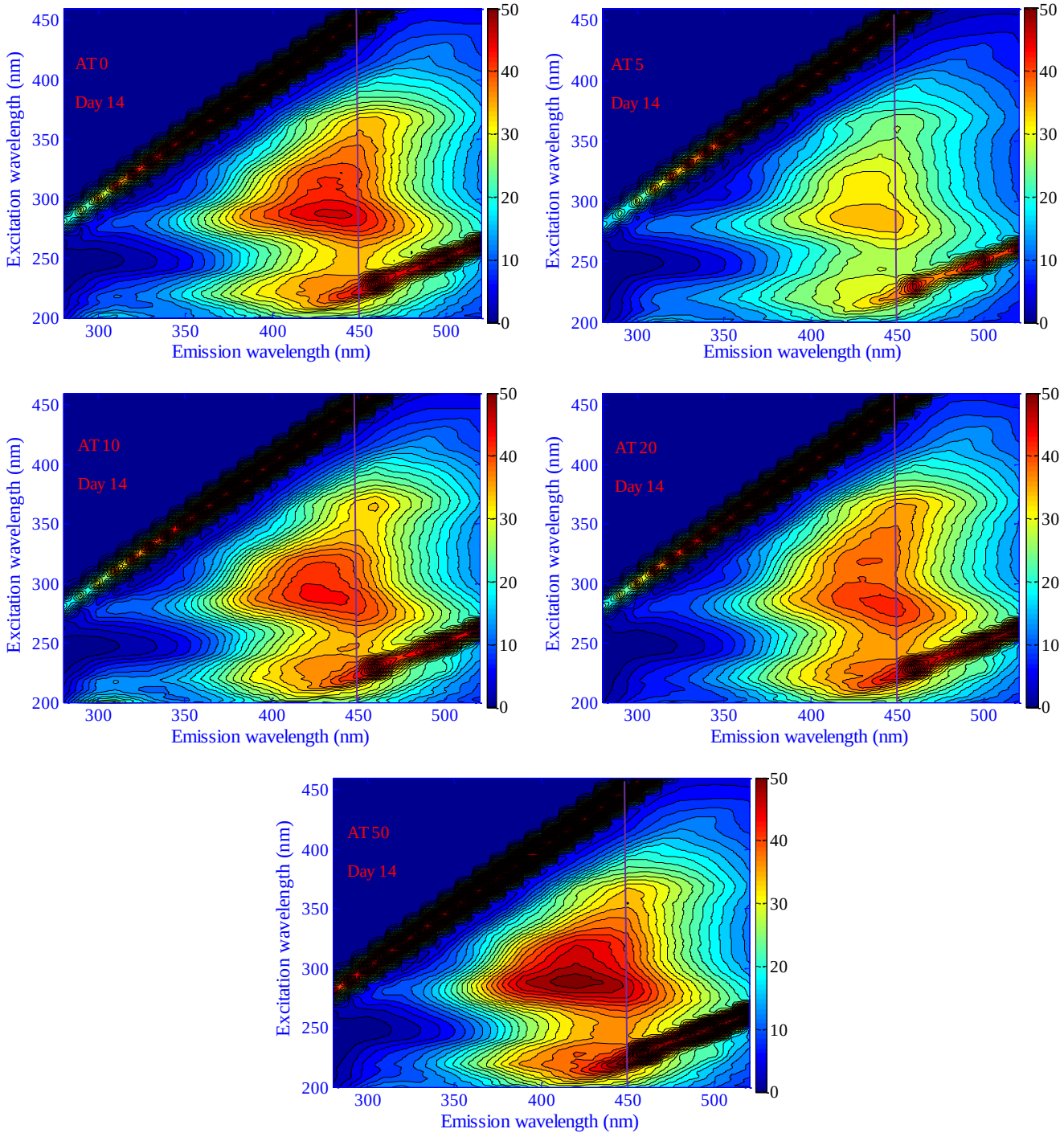

Fig 3-20. EEM contour maps of day 14 for the different experiments (AT 0 through AT 50) conducted using Ag-TiO_2-NPs.

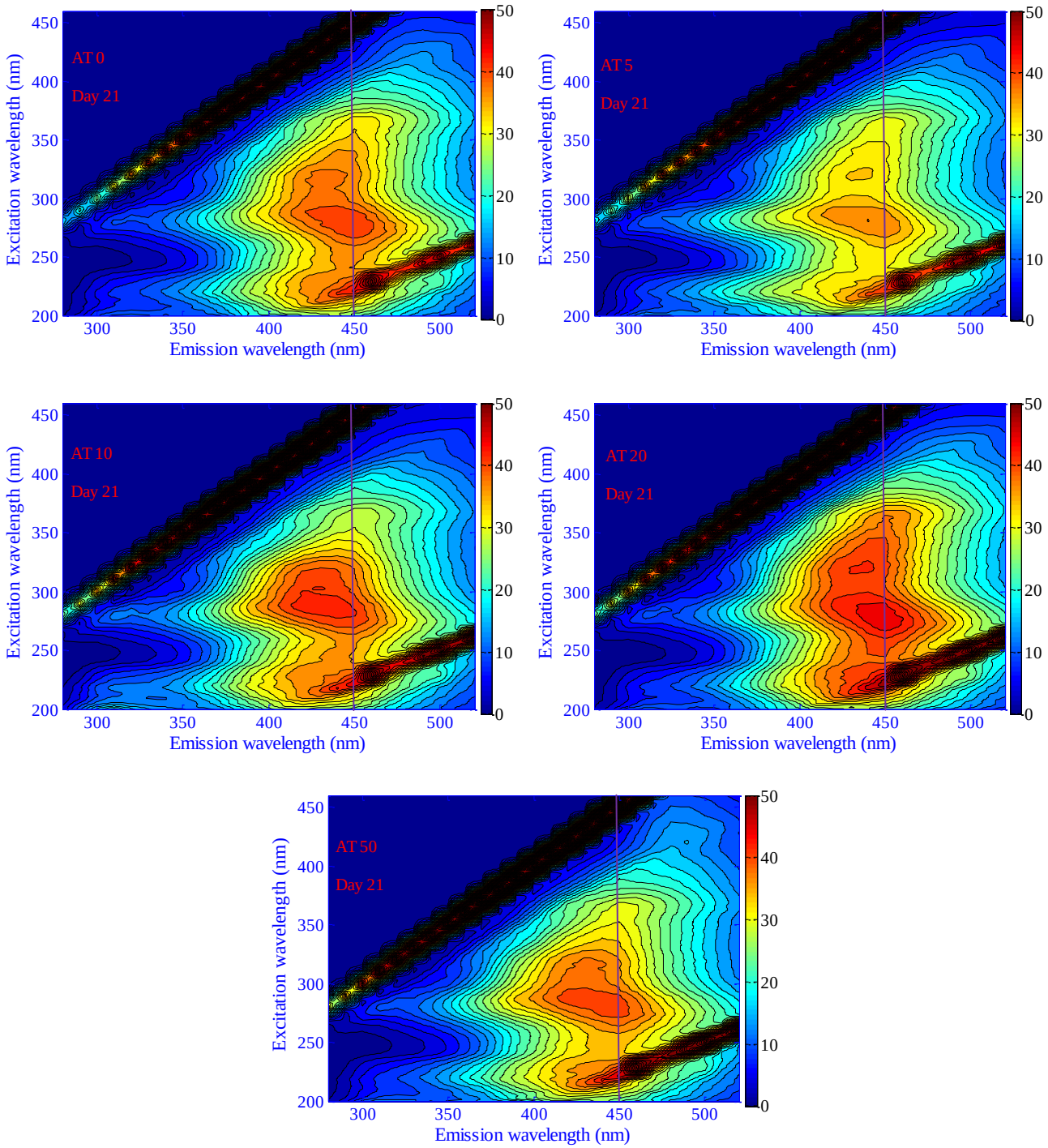

Fig 3-21. EEM contour maps of day 21 for the different experiments (AT 0 through AT 50) conducted using Ag-TiO_2-NPs.

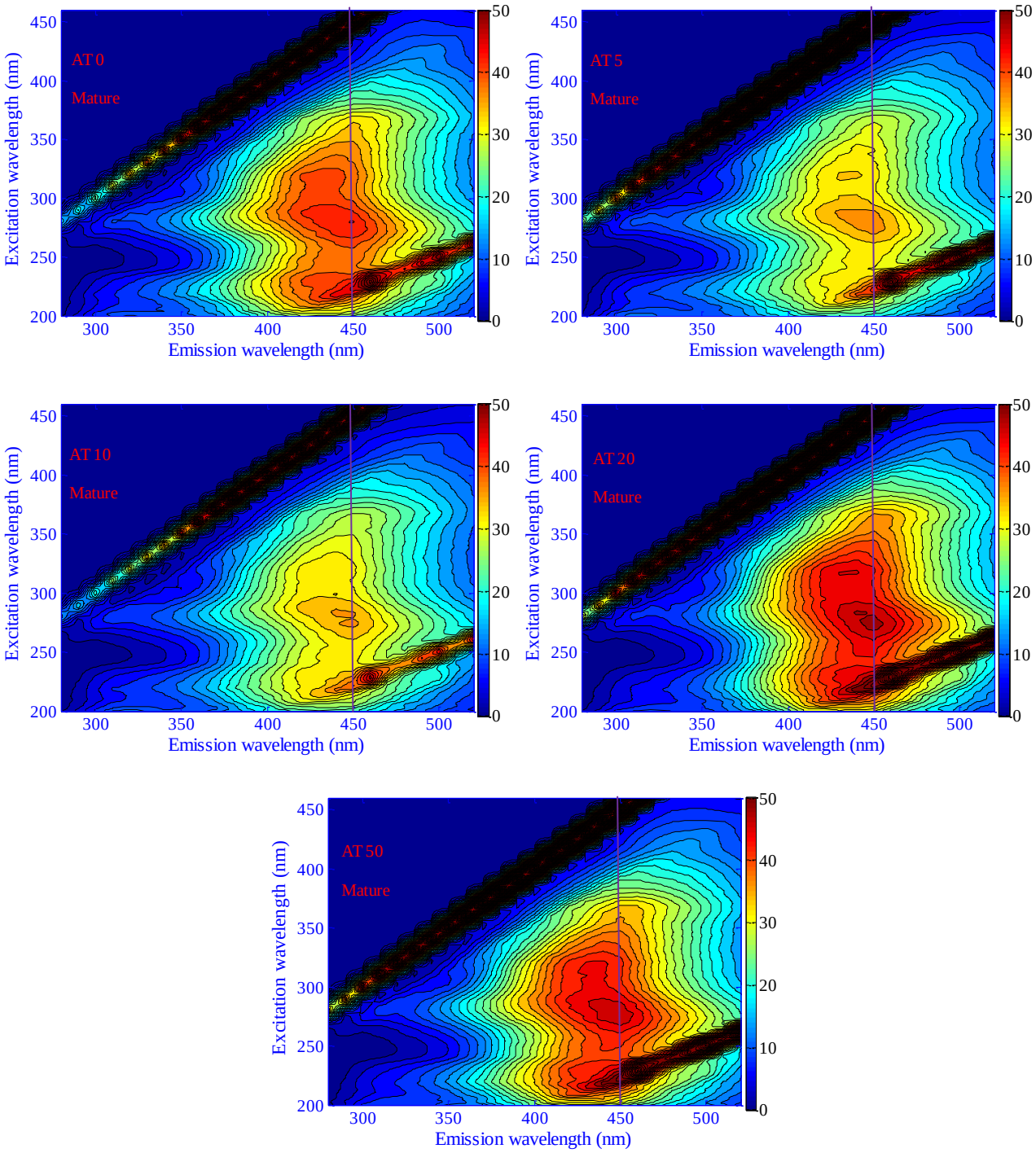

Fig 3-22. EEM contour maps of mature samples for the different experiments (AT 0 through AT 50) conducted using Ag-TiO_2-NPs.

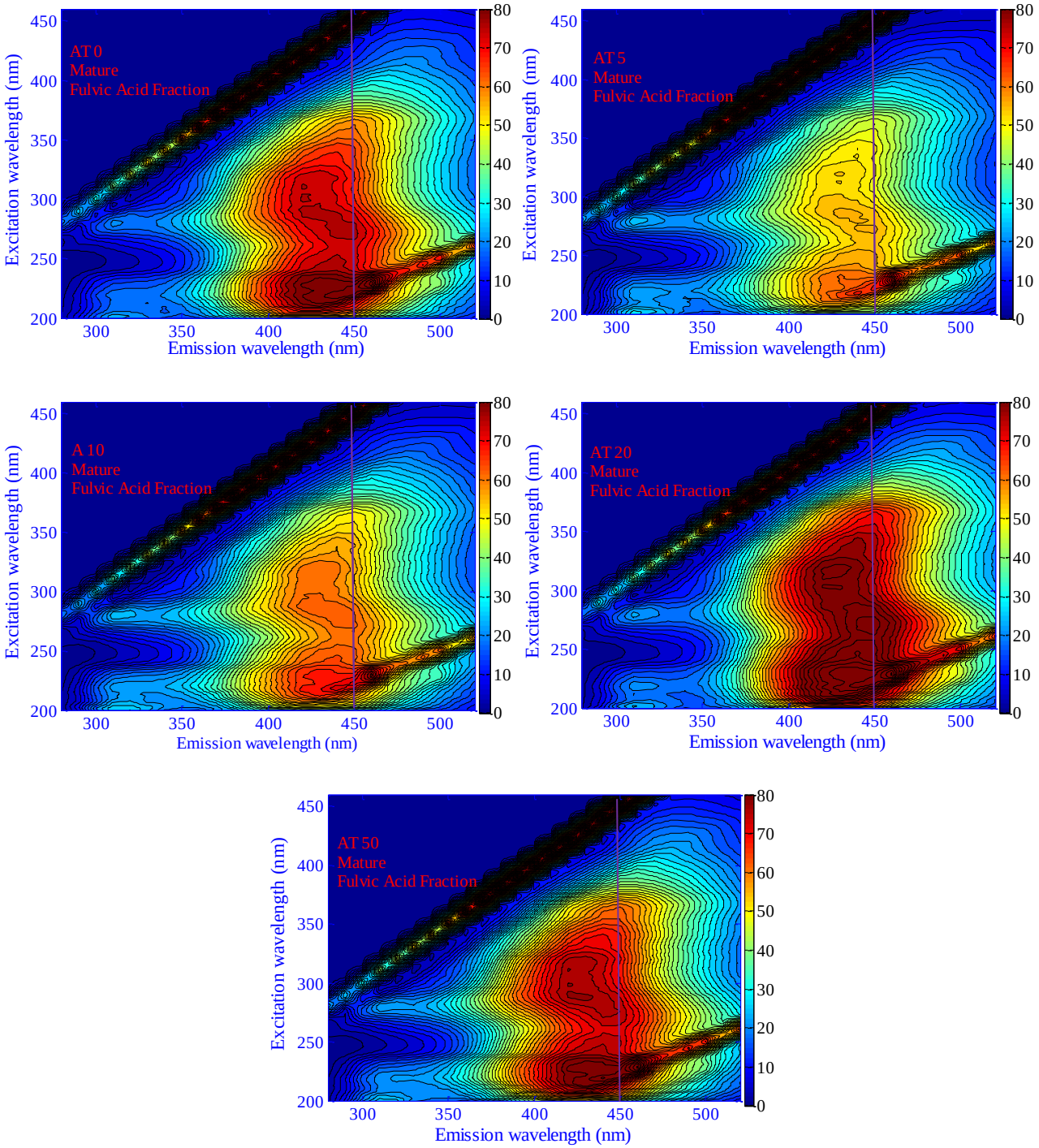

Fig 3-23. EEM contour maps of FA fraction derived from mature samples for the different experiments (AT 0 through AT 50) conducted using Ag-TiO_2-NPs.

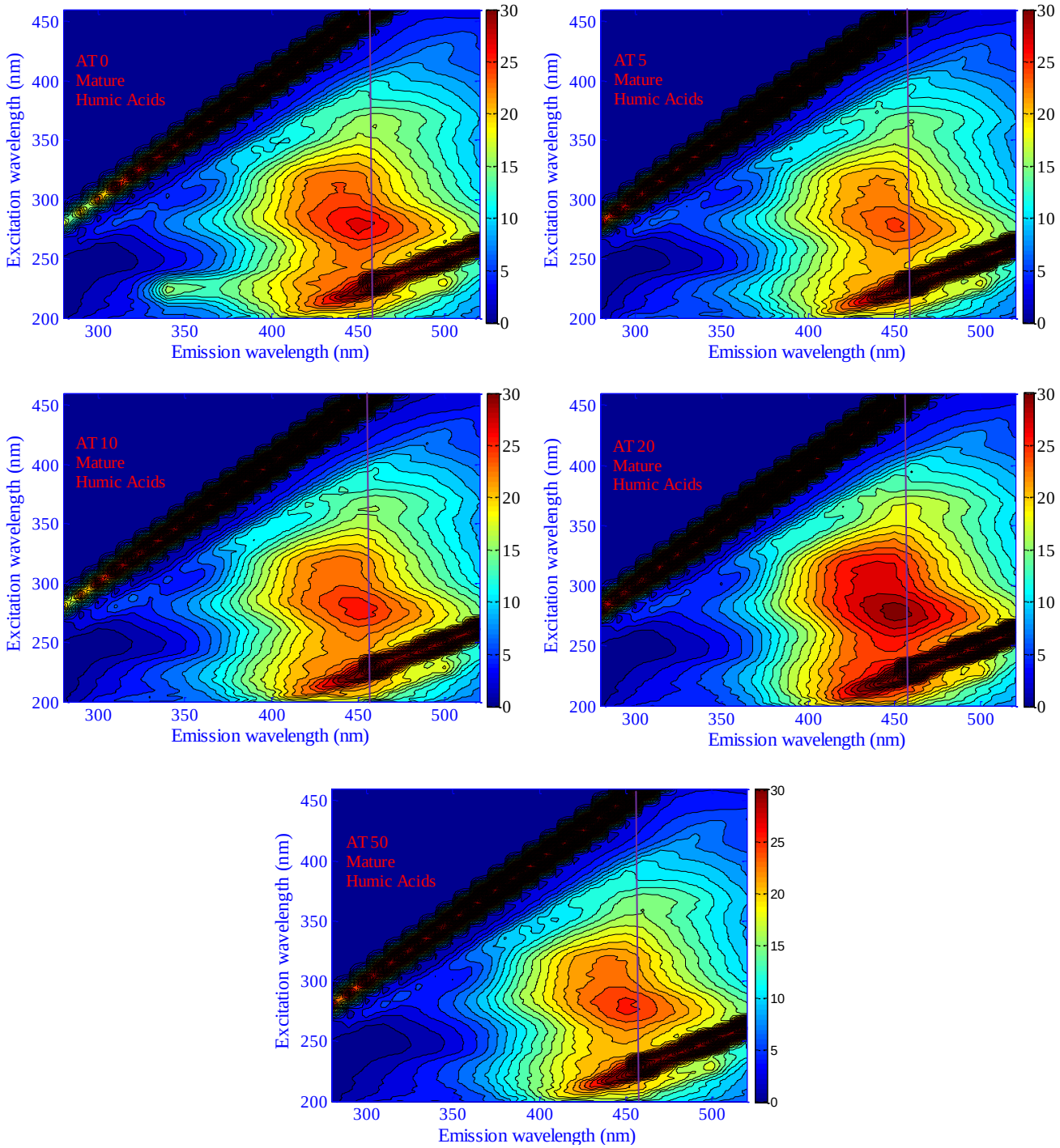

Fig 3-24. EEM contour maps of HA derived from mature samples for the different experiments (AT 0 through AT 50) conducted using Ag-TiO_2-NPs.

3.1.5.5 Organic waste composting contaminated with AgNPs

The compost pH values for the different experiments: A 0; A 5; A 10; A 20, A 50 and matrix (see sub-chapter 3.1.3.2 for explanation) are given in Figure 3-25. The initial pH of composting mixtures ranged between 5.67-5.85 and then increased to 6.92-7.20 at day 4. The pH values further increased to 7.20-7.84 at day 7 for all experiments and then remained at the same levels for the remaining length of the treatment (21 days), apart from experiment A 50 which at day 14 presented a higher pH value as compared to the rest of the conducted experiments.

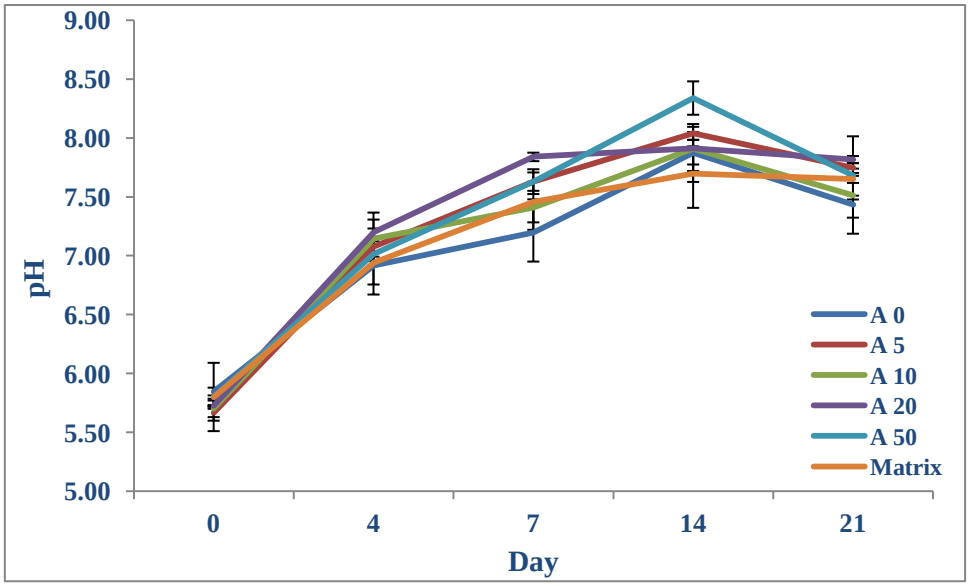

Fig 3-25. The pH values for the different experiments conducted using AgNPs.

The MC values are given in Figure 3-26. The MC were kept at the same levels for all the experiments and ranged between 68.29-77.69%.

The ash content values are given in Figure 3-27. The initial ash content was between 5.07-5.12% for all the experiments. At day 4, the ash content increased to 6.56-6.79% for all the experiments apart of A 50 where the ash content increased to 7.21% slightly higher from the rest of experiments and the matrix where the ash content increased to 6.11% slightly lower from the rest of experiments. Similar results were obtained for days 7, 14 and 21. Also, in this set of experiments, it is clear that the 1st week of composting the ash production was higher compared to the 2nd and 3rd week.

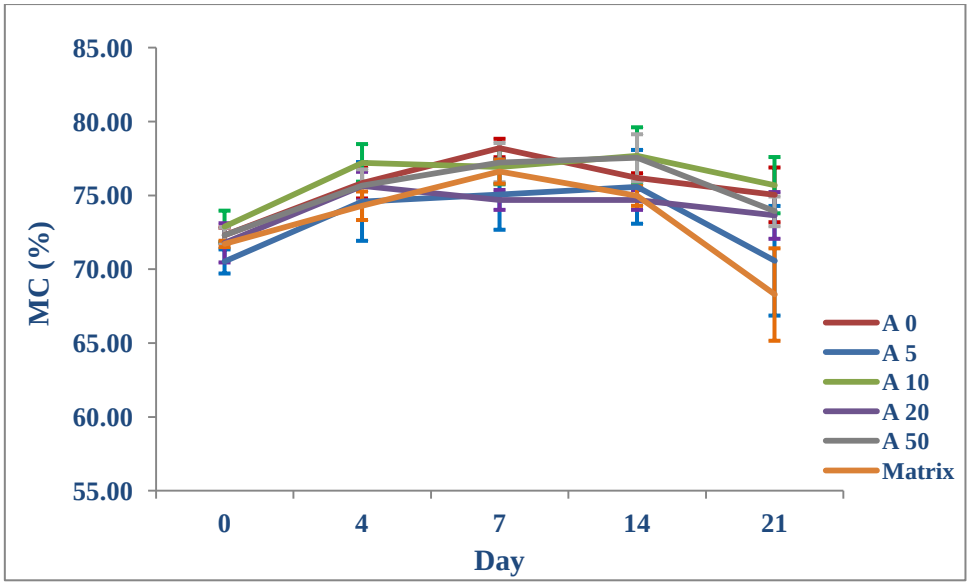

Fig 3-26. MC values for the different experiments conducted using AgNPs.

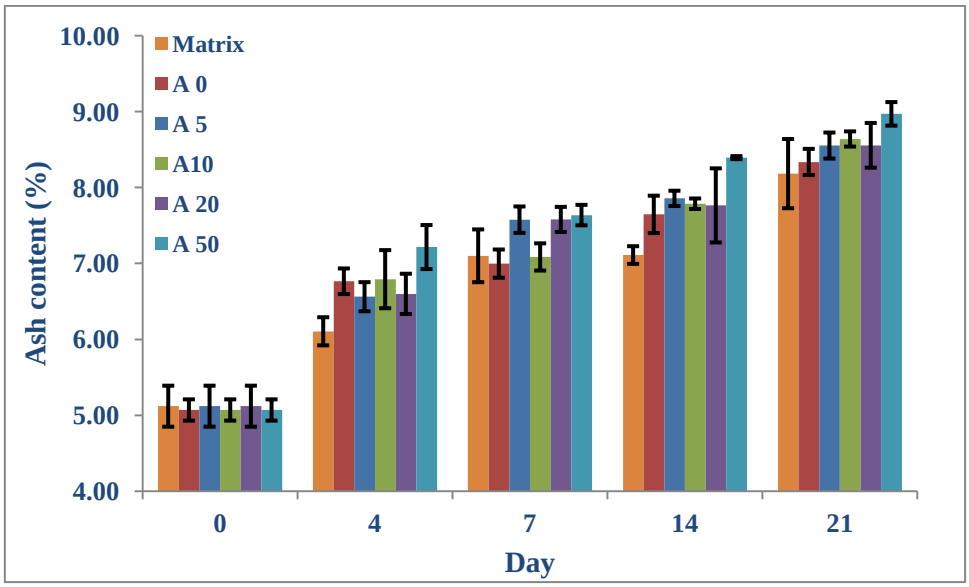

Fig 3-27. Ash content percentage for the different experiments conducted using AgNPs.

The weight on a dry basis and the percentage of the weight loss following 21 days of continuous treatment are given in Table 3-10 and Figure 3-28. The percentage of weight loss was similar for the experiment A 0, A 50 and matrix; a higher standard deviation for treatment A 50 was observed. The experiment A 10 showed the highest percentage of weight loss followed by experiments A 5 and A 20, which showed a relative higher standard deviation than the former.

Table 3-10. Dry weight and % weight loss of waste after 21 days of in-vessel composting.

	Dry weigth (g)		(%) Loss	
	Av	**StDev**	**Av**	**StDev**
A 0	15.92	0.17	41.58	0.65
A 5	15.40	0.59	43.49	2.18
A 10	14.21	0.41	47.83	1.53
A 20	15.20	0.60	44.22	2.19
A 50	15.96	0.75	41.43	2.78
Matrix	15.77	0.82	42.13	3.02

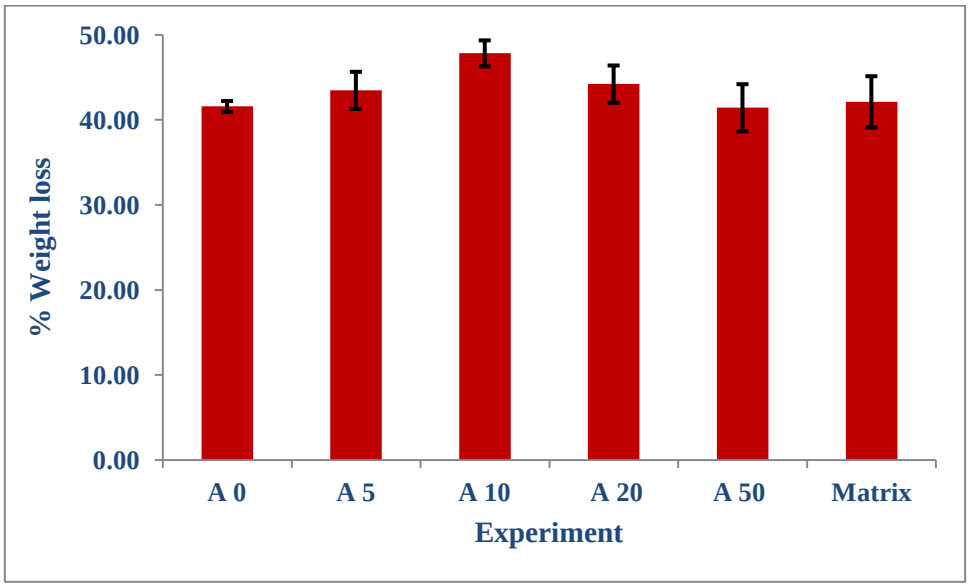

Fig 3-28. Percentage of weight loss of waste after 21 days of in-vessel composting.

A qualitative analysis (see sub-chapter 3.1.4.3) using EEM fluorescence spectroscopy was performed to monitor the formation of HS.

EEM spectra obtained for the different treatments at day 4 are presented in Figure 3-29. The matrix treatment appeared to have the lower decomposition rate because it exhibited peaks at Ex/Em 220/320, 280/320 and 220/360 nm/nm that are absent in the rest of the treatments. Treatment A 10 appeared to have the highest decomposition rate as suggested by the peaks shifting towards emission wavelengths higher than 400 nm while the rest of the samples exhibited peaks with emission wavelengths lower than 400 nm.

EEM spectra obtained for the different treatments at day 7 are presented in Figure 3-30. At day 7, all the samples exhibited peaks in the HS region. The peaks associated with HS suggest that HS are in the first stages of formation with FA and HA

associated peaks very close to each other, therefore the results obtained at day 7 re not conclusive (see Appendix A 1.2).

EEM spectra obtained for the different treatments at day 14 are presented in Figure 3-31. At day 14, A0, A 20 and A50 treatments appeared to have the lowest decomposition rates as suggested by the broader region of the HS which include peaks in emission wavelength as low as 420 nm. Treatment A 10 appeared to have the highest decomposition as suggested by a narrow HS region and lower intensities. The lower intensity in the HS region at this stage indicates the formation of HA from the FA. FA exhibited higher intensities as compared to HA, which correlates with lower molecular weights. If a sample contains higher concentration of HA as compared to FA then the HA peaks results in lower peak intensities as peak intensity decreases when the molecular weight increases. It was expected that the peak intensities in the HS region would be lower through the decomposition process but this can be complex because the decomposition process is in progress and the formation of FA and HA is continuing.

EEM spectra, at day 21 and for mature samples, obtained for the different treatments are presented in Figures 3-32 and 3-33, respectively. It was observed that at day 21 FA and HA peaks overlapped, and therefore no conclusions could be drawn (See Appendix A 1.2). In an attempt to better characterise FA and HA peaks, FA fraction was separated from HA (see sub-chapter 3.1.4.3) and different EEM spectra obtained. EEM spectra for the FA fraction of mature samples are presented in Figure 3-34. A0 and A10 exhibited the higher intensities for the FA fraction. All the spectra exhibited peaks in the API and SM region at Ex/Em 220/310 and 280/310 nm/nm. Those peaks represent the remaining OM available for degradation. The intensities of those peaks were highest for A 5, A 20, A 50 and Matrix and lowest for A 0 and A 10. This may indicate a higher decomposition rate for A 0 and A 10 in terms of the formation of FA from lower molecular weight compounds. The FA concentrations are given in Table 3-11 (See also Appendix A 1.6).

EEM spectra of isolated HA from mature samples are presented in Figure 3-35. A 5 exhibited the highest peak intensities for HA while the other treatments exhibited similar peak intensities in the EEM spectra. The HA concentrations are given in Table 3-12 (See also Appendix A 1.5).

Table 3-11. The concentration of FA in mg/g for the different treatments.

Sample	**A 0**	**A 5**	**A 10**	**A 20**	**A 50**
	mg/g	**mg/g**	**mg/g**	**mg/g**	**mg/g**
1	10.96	9.95	10.64	7.99	8.82
2	12.63	8.34	9.57	8.36	9.60
3	10.96	10.75	11.97	9.43	11.89
Av	**11.52**	**9.68**	**10.73**	**8.59**	**10.10**
StDev	0.96	1.23	1.20	0.75	1.60

Table 3-12. The concentration of HA in mg/g for the different treatments.

Sample	**A 0**	**A 5**	**A 10**	**A 20**	**A 50**
	mg/g	**mg/g**	**mg/g**	**mg/g**	**mg/g**
1	10.36	10.79	10.21	11.10	9.74
2	11.10	n/a	10.46	9.36	10.26
3	10.32	11.15	10.88	9.65	11.21
Av	**10.59**	**10.97**	**10.52**	**10.04**	**10.41**
StDev	0.44	0.25	0.34	0.93	0.74

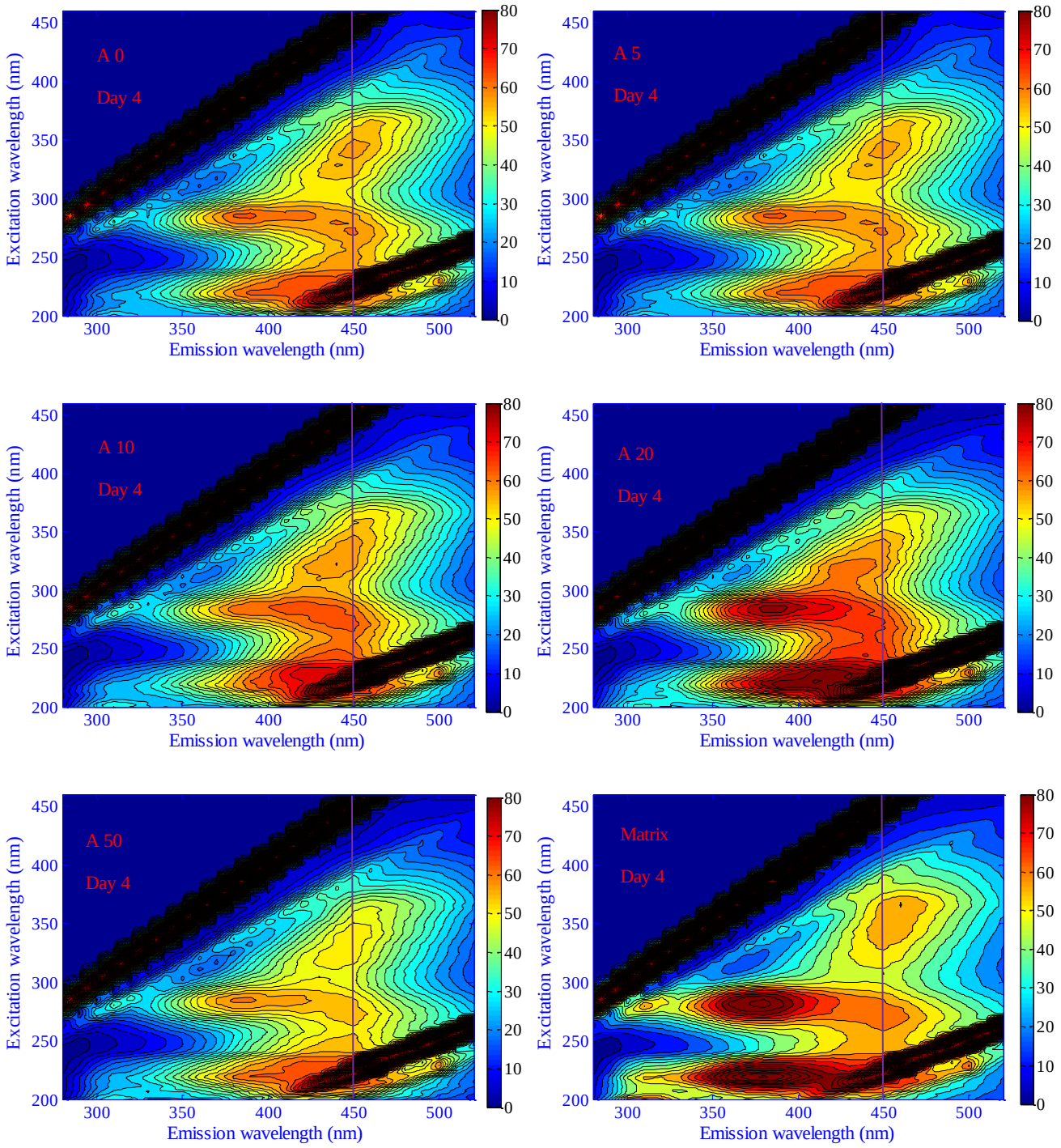

Fig 3-29. EEM contour maps of day 4 for the different experiments conducted using AgNPs.

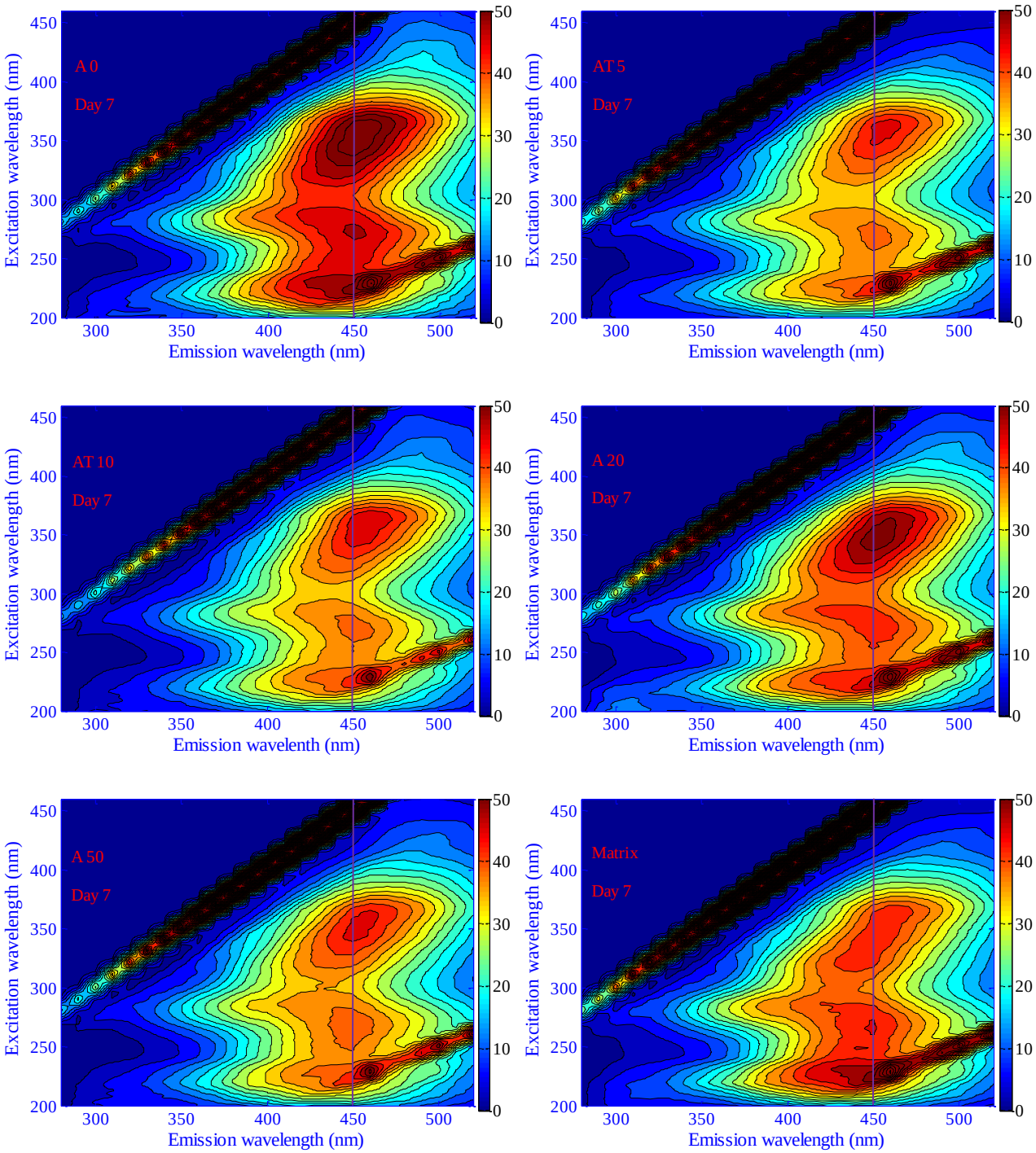

Fig 3-30. EEM contour maps of day 7 for the different experiments conducted using AgNPs.

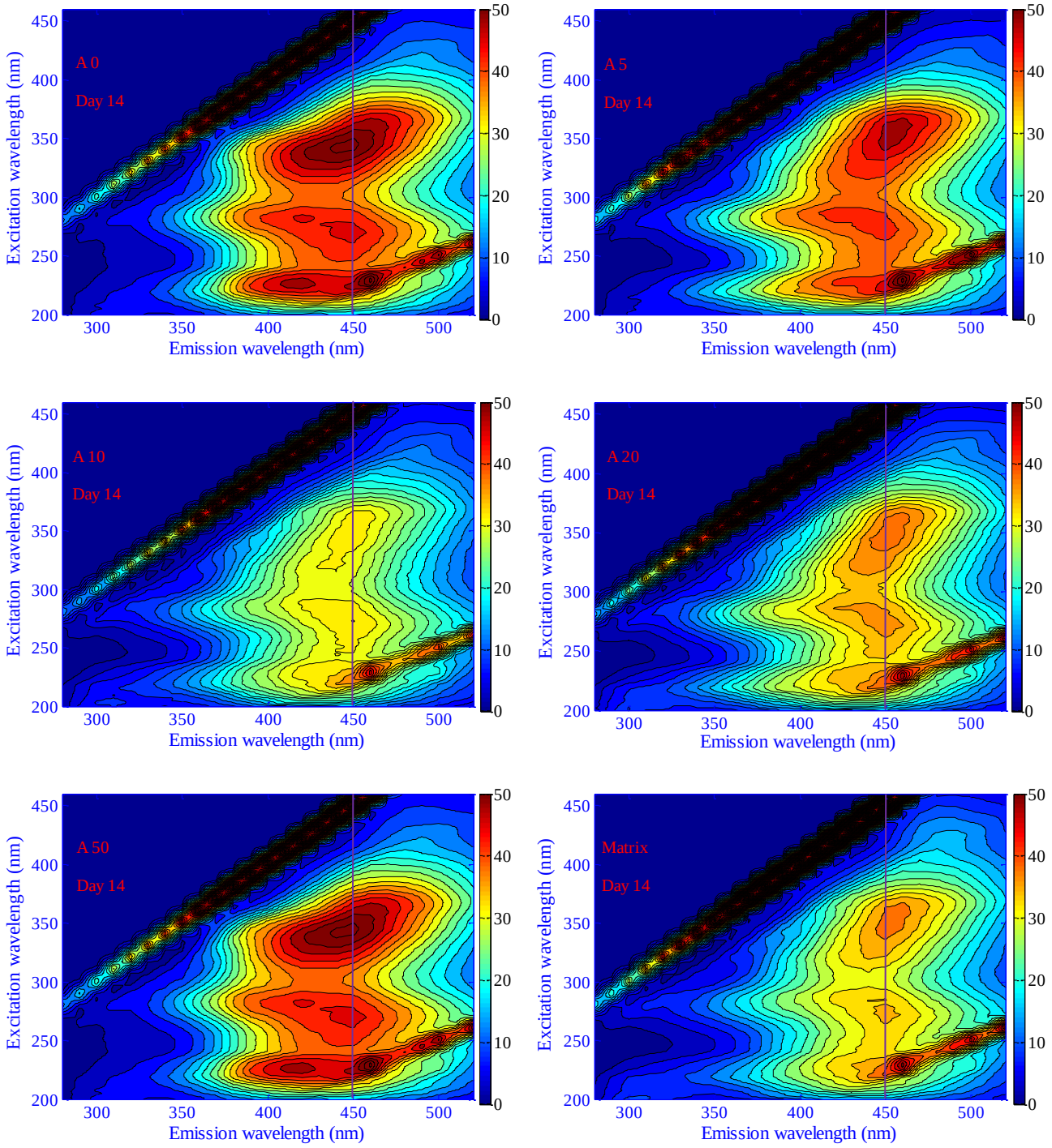

Fig 3-31. EEM contour maps of day 14 for the different experiments conducted using AgNPs.

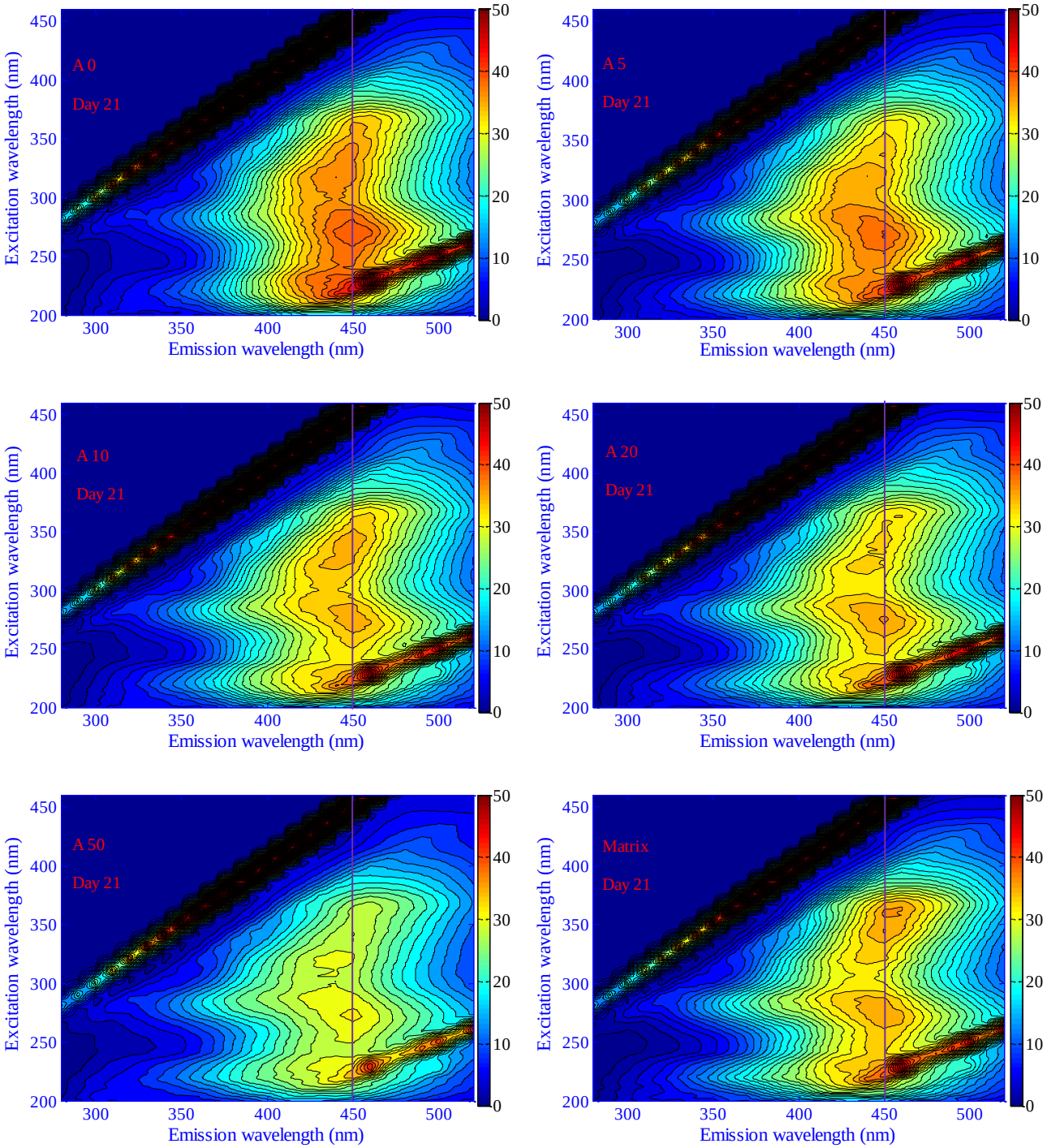

Fig 3-32. EEM contour maps of day 21 for the different experiments conducted using AgNPs.

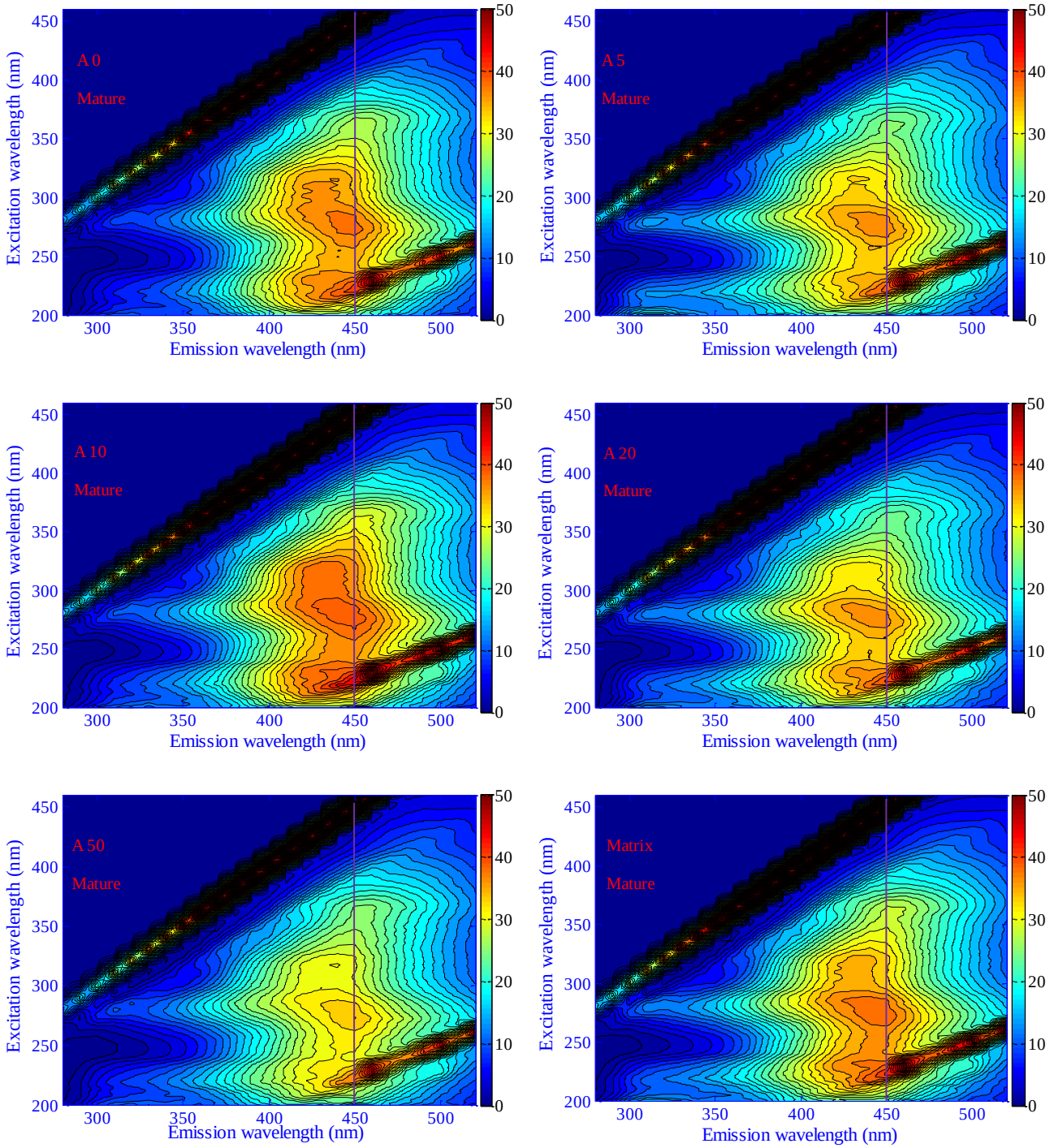

Fig 3-33. EEM contour maps of mature samples for the different experiments conducted using AgNPs.

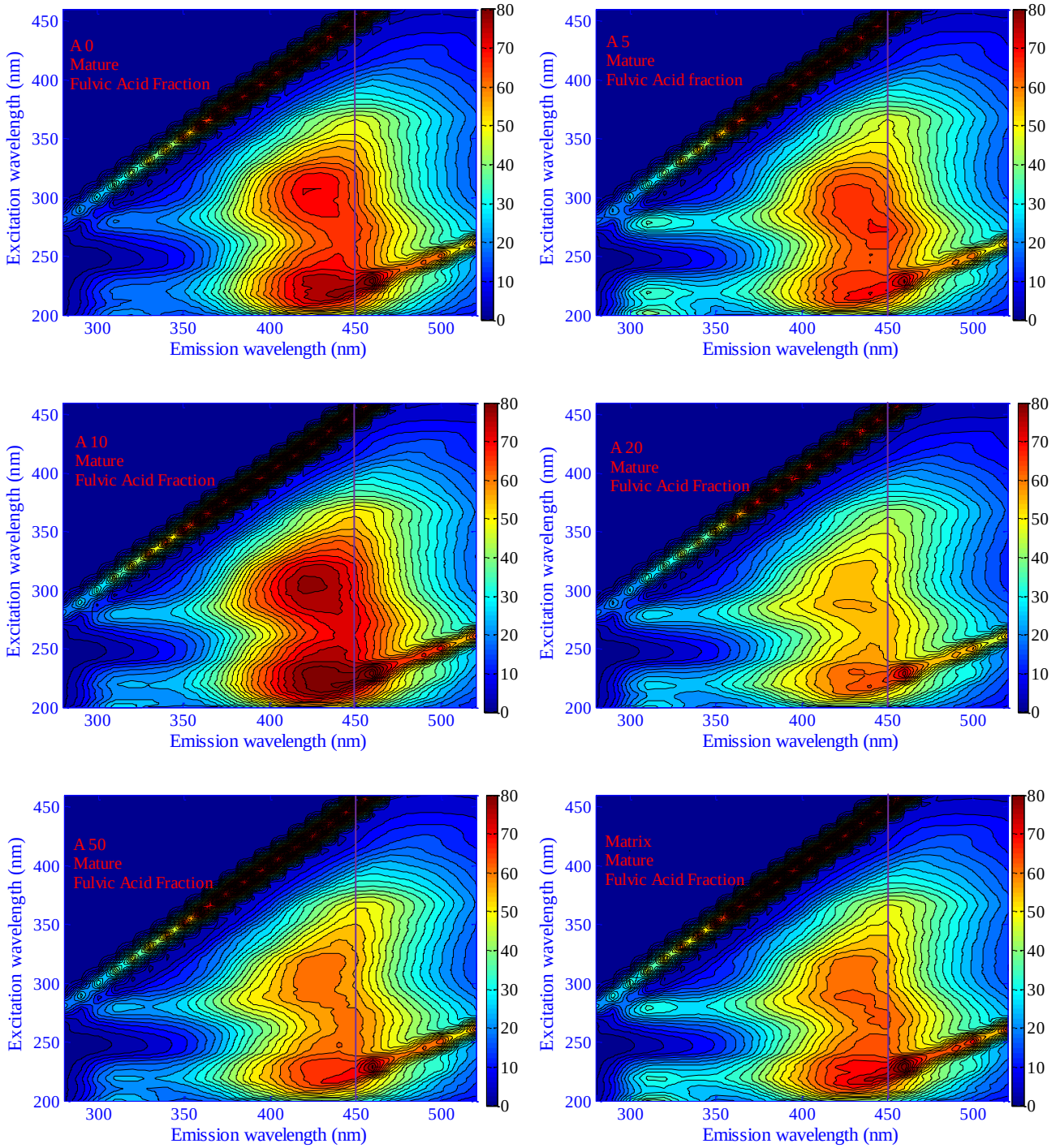

Fig 3-34. EEM contour maps of FA fraction derived from mature samples for the different experiments conducted using AgNPs.

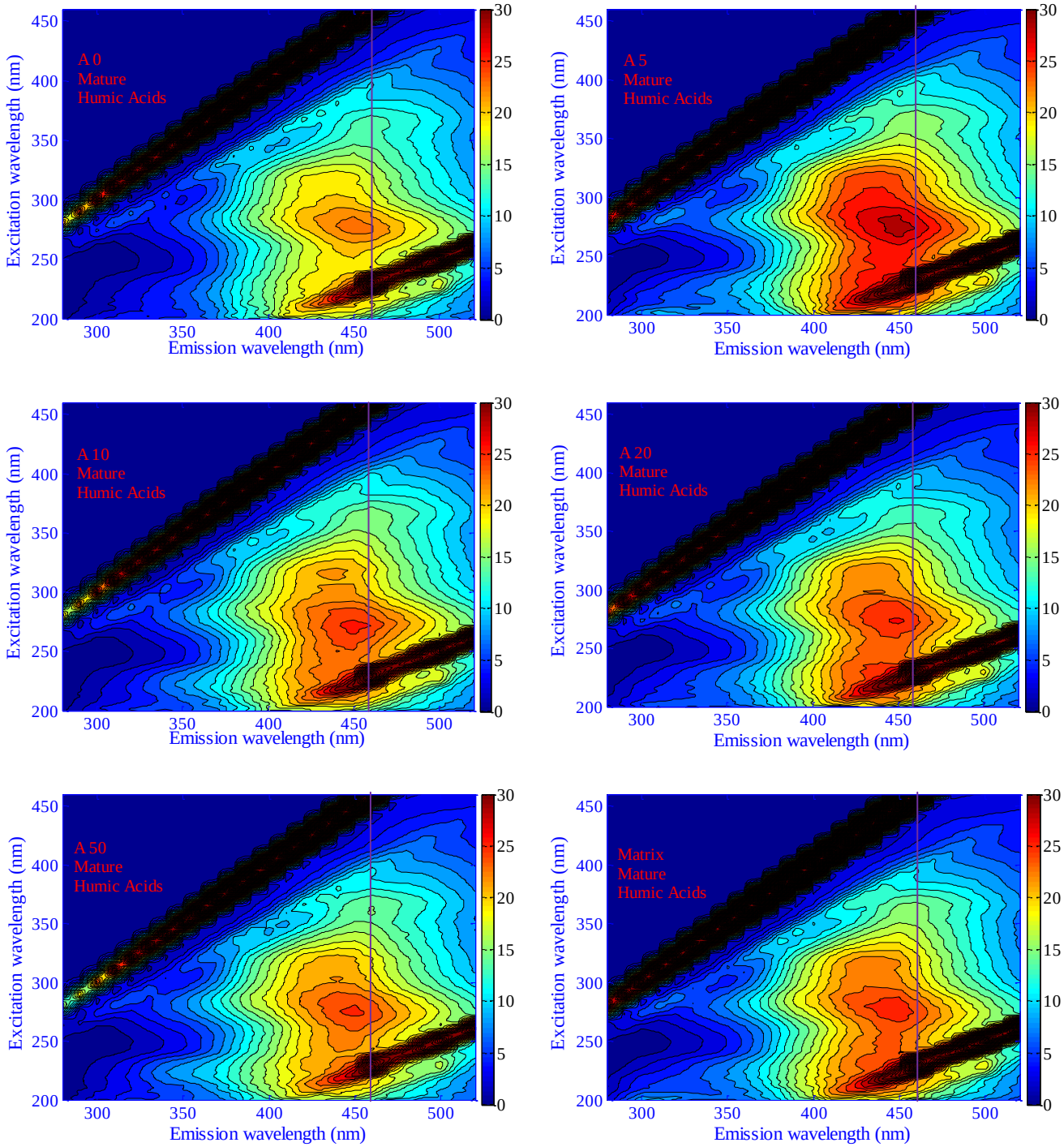

Fig 3-35. EEM contour maps of HA derived from mature samples for the different experiments conducted using AgNPs.

Ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N) and phosphorus (P) values are given in Table 3-13 and Figures 3-36, 3-37, 3-38 and 3-39 respectively.

Table 3-13. Available ammoniacal-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N) and phosphorus from mature compost.

sample	NH_4^+-N(mg/kg)		NO_3^--N (mg/kg)		NO_2^--N (mg/kg)		P (mg/kg)	
	Av	StDev	Av	StDev	Av	StDev	Av	StDev
A 0	83.05	4.55	0.352	0.081	0.046	0.008	855.60	116.81
A 5	64.26	1.04	0.739	0.003	0.043	0.003	1010.24	43.98
A 10	79.83	4.81	0.824	0.041	0.054	0.021	1155.24	46.28
A 20	62.37	7.22	0.343	0.092	0.048	0.012	1182.96	117.15
A 50	57.35	4.93	0.278	0.039	0.055	0.008	1172.53	42.77

The concentration of NH_4^+-N was higher for A 0 and A 10 than for the rest of the treatments. A 5, A 20 and A 50 treatments exhibited similar values. The concentration of available NO_3^--N was highest for A 5 and A 10 while for A 0, A 20 and A 50 was much lower with no great differences between them. The concentration of the available NO_2^--N was very low with no substantial differences between treatments. The concentration of available phosphorus was lowest for A 0, slightly increased for A 5 and A 10 and then remained at similar levels for A 10, A 20 and A 50.

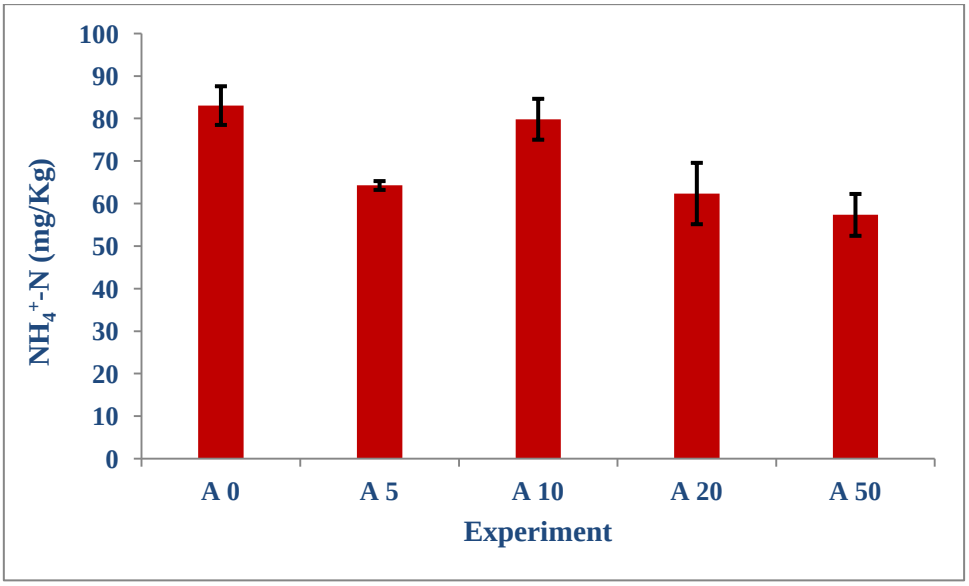

Fig 3-36. Available ammoniacal-nitrogen (NH_4^+-N) from mature compost.

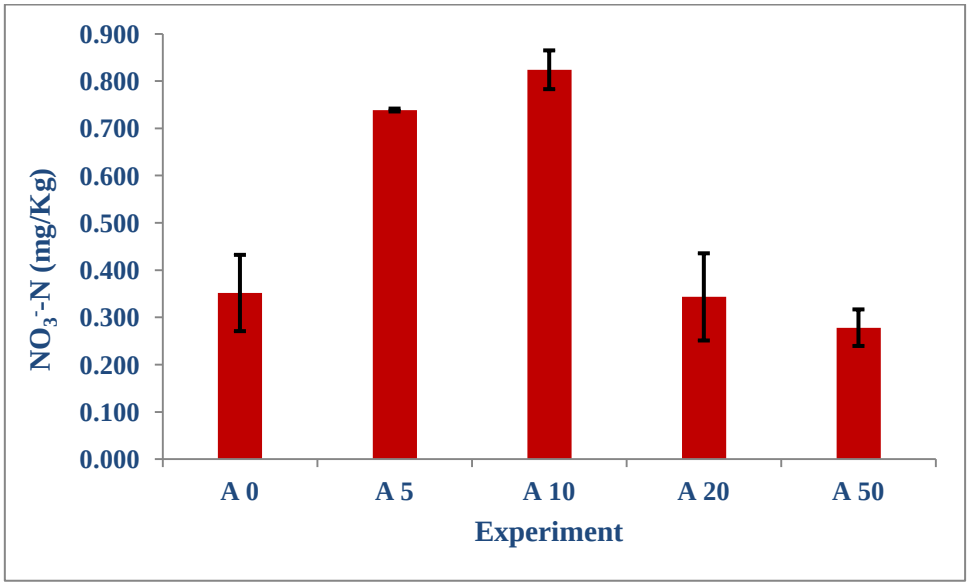

Fig 3-37. Available nitrate-nitrogen (NO_3^--N) from mature compost.

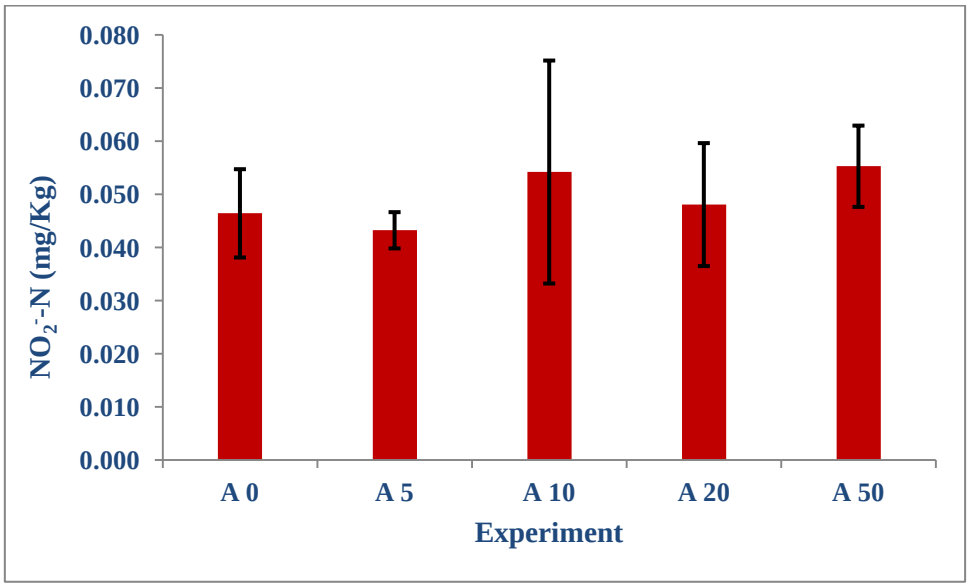

Fig 3-38. Available nitrite nitrogen (NO_2^--N) from mature compost.

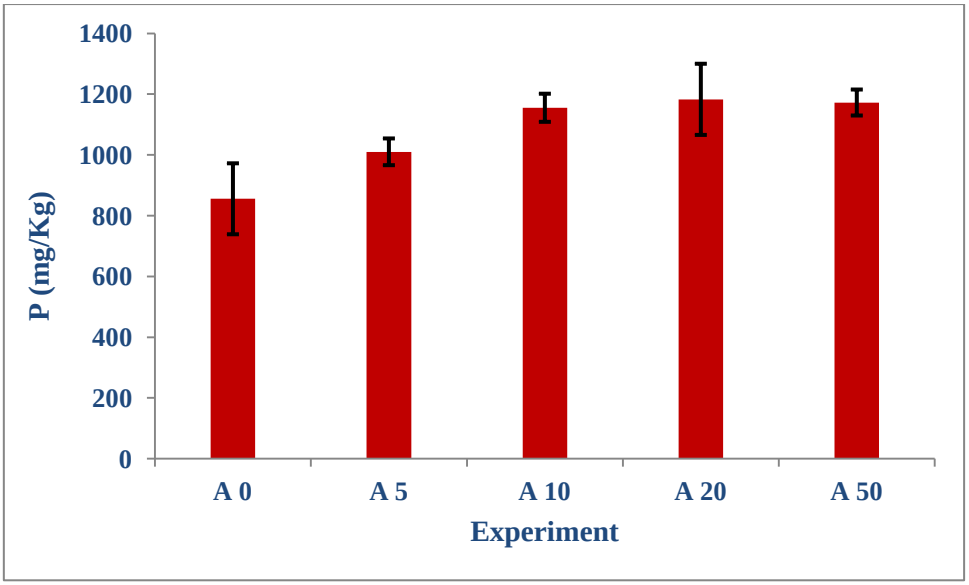

Fig 3-39. Available phosphorus from mature compost.

3.1.6 Discussion

The results indicated that the formation of HA was highest for AT 20 but the ash content was highest for AT 50, when all treatments were compared. It has been reported that the formation of HA may indicate the evolution of the mineralization process but this was not observed in the presence of NPs here. The accumulation of humins in nature slows the mineralization of OM because this heterogeneous mixture require either a very large assemblage of enzymes or versatile enzymes to affect its rapid mineralization (Rice 2001). This may imply that the formation rate of humins was highest for AT 0 treatment and it decreased as the concentration of Ag-TiO_2-NPs in waste increased. This may explain why carbon mineralization was lowest for AT 0 and highest for AT 50.

The results indicated that the formation of HA was highest for A 5 but the ash content was highest for A 50. This may imply that the formation rate of humins was highest for A 0 and it decreased as the concentration of AgNPs in waste increased. This may explain why carbon mineralization was lowest for A 0 and Matrix and highest for A 50.

The peaks intensity of diluted compost extracts increased at the early composting stages and then decreased with increasing time of composting. This result is in agreement with the findings of Provenzano et *al.* (2001). The peaks intensity provides useful information on the evolution of OM during composting. The initial peaks intensity increase may be due to the increased probability of electron transition

between the singlet and ground state, which occurs when decomposition occurs (Provenzano, de Oliveira et al. 2001). It is necessary to differentiate between diluted sample peak intensity and undiluted sample peak intensity. The peak intensities of undiluted samples increase with time of composting because the dilution factors are higher with time. The peak intensities of the diluted sample decreased here and this was attributed to the protocol analysis. According to the protocol analysis, the samples were diluted to have a value of 0.3Å when the UV absorbance at λ=254 nm was measured. The continuous HS formation and the shift of the peaks towards higher Ex/Em wavelengths with time resulted in higher molecular weight compounds (HA) that exhibited lower peak intensities compared to low molecular weight compounds such as aromatic proteins. It was suggested that the decrease of peaks intensity with composting time was due to the higher percentage of HA present in compost extract.

Fluorescence spectroscopy has been used as a non-destructive tool to quantify the compost humification and stability. It has been suggested that the formation of HS can be monitored, using EEM fluorescence spectroscopy, which provides evidence of progressive mineralisation and humification of the composting mixture (Antizar-Ladislao, Lopez-Real et al. 2006). The shift of the peaks towards the HS region during composting for all the treatments indicated that NPs did not appear to have an effect in humification and therefore in compost stability. The weight loss percentage, on dry weight, was *ca.* 40% during composting of all treatments which implied the good performance of the method especially when comparing with NaN_3 treatment where the weight loss percentage was 2.88%. The results indicated no significant differences on availability of NH_4^+-N, NO_2^--N and P from AgNP-contaminated mature compost samples while the availability of nitrate was higher for A 5 and A 10.

The main hypothesis of the thesis was that NPs present in waste will have an impact in composting process due to their antibacterial properties but it was rejected as composting process exhibited a very good performance. The overall bacterial community structure has been reported to change during composting of AgNP-contaminated MSW at an initial concentration of 2 mg Ag / kg compared with non contaminated MSW composting. The changes had no effect to the functional performance of composting process probably due to the potential functional redundancy within the compost samples (Gitipour, El Badawy et al. 2013). It has been reported that NP toxicity relates to the size, shape and individuality of NPs in solution because small NPs (< 10 nm) may pass through cell membranes, accumulate and lead

to cell malfunction (Choi, Deng et al. 2008). The presence of HA has been reported to decrease the size of NPs and the same time to reduce their toxicity instead of increase this. It has been suggested that the toxicity was mitigated due to the sorption of HA on the NP surface and consequently changes on the surface properties of the particle (Fabrega, Fawcett et al. 2009).

3.1.7 Final remarks

The use of NaN_3 as an inhibitor of microbial activity in biodegradable MSW effectively inhibited the microbial activity the first week of composting. The microbial population activated the second week of composting but the decomposition rate was so low that did not result in the formation of HS following 21 days of composting. Both treatments, using Ag-TiO_2-NPs and AgNPs, did not appear to show any inhibition of the decomposition process for all the tested concentrations and the peaks shifted towards the HS region. Higher carbon mineralization rates resulted for NP-contaminated compost with the higher NP concentrations. This may indicate that the formation of humins is higher for non-contaminated compost and decreases as the NP concentration in waste increases. The results indicated no significant differences on availability of NH_4^+-N, NO_2^--N and P from AgNP-contaminated mature compost samples while the availability of nitrate was higher for A 5 and A 10.

3.2 Environmental Impact of Compost obtained from Nanoparticles – Contaminated Biodegradable Municipal Solid Waste

3.2.1 Introduction

Soil organic matter refers to the whole of organic material in soils and has often partitioned in active (i.e. plant litter, light fraction, non humic substances) and stable (i.e. passive humus) (Bare, Norris et al. 2002). Active fraction serves as a ready source of nutrients (N, P and S) for plant growth while stable fraction functions as a reservoir of plant nutrients and is important from the long-term balance of the soil (Stevenson 1994). The proper management of soil OM is of particular importance to achieve sustainable agriculture. Compost application in soils is the most common input OM in agriculture to replace the OM losses and consequently increase soil productivity (Murray, Pinchin et al. 2011).

The fractions of heavy metals that can be readily mobilized in the soil environment and taken up by plant roots are considered the bioavailable fraction. The bioavailability has been defined as the extent to which a chemical can be absorbed by a living organism and reach the systemic circulation. The total metal concentration in soil does not necessarily correspond with metal availability. The availability of heavy metals to plants depend on a number of physical and chemical factors in the soil such as pH, organic matter content, redox potential and cation exchange capacity.

Bioavailable and bioaccessible are here defined to clarify the differences between both terms and associated implications (Semple, Doick et al. 2004). While bioavailable implies immediacy, bioaccesible implies that some of what is available can be reached but is often not within reach at a given time from a given place. Hence, according to Semple *et al.* (2004) a bioavailable compound is defined as '*that which is freely available to cross an organism's cellular membrane from the medium the organisms inhabits at a given time*', while a bioaccessible compound is defined as '*that which is available to cross an organism's cellular membrane from the environment, if the organisms have access to the chemical*'.

One of the most important factors governing solubility and bioavailability of metals in soil-plant systems is organic complexation of metals. The organic compounds in soil which could form metal complexes can be divided into three main classes: (a) naturally occurring soil organic molecules of known structure and chemical properties

(aliphatic acids, polysaccharides, amino acids and polyphenols), (b) antropogenically derived organic chemicals from agriculture, industrial and urban activities and (c) humic substances. OM decomposition continually changes the composition of soil OM (Senesi 1992). The composition of soil OM is dominated by humins, HA and FA. Intawongse *et al.* (2006) observed that individual plant species (lettuce, spinach, radish and carrot) greatly differ in their metal (Cd, Cu, Mn and Zn) uptake from contaminated compost (Intawongse and Dean 2006). Depending on the type and the quality of compost, compost amendments are believed to immobilize metals resulting in lower toxicity.

Many countries have introduced compost guidelines regulating compost quality. The use of compost in agriculture in UK is controlled under PAS100 which is a technical report for the production and use of compost from source segregated bio waste (Agency 2007).

Leaching is the process by which soluble constituents are dissolved from a solid material (such as rock, soil or waste) into a fluid by percolation or diffusion. The soluble constituents dissolved in the liquid form a "leachate". The capacity of the material to leach is called its "leachability". Several factors such as chemical (pH, redox potential, polarity, temperature etc.), physical (particle size, heterogeneity, surface area, liquid to solid ratio etc.), biological and the length of the time affect the leachability of the material. The pH is the most important parameter influencing metal release from fly ash during leaching (Brunori, Balzamo et al. 1999).

3.2.2 Aims and objectives

The main aim of this study is to assess the environmental impact of compost obtained from NP-contaminated biodegradable MSW.

The objectives of this study are as follow:

(a) to investigate the phytotoxicity of NP-contaminated compost using a seed germination bioassay,

(b) to investigate the leaching potential and properties of NP-contaminated compost using a column leaching experiment.

3.2.3 Materials and Methods

3.2.3.1 Materials

Mature compost was used for the experiments. The compost was grinded to pass a 2 mm sieve. Ag and Ti concentrations in compost were higher than those concentrations in the original waste mixture because of the weight loss following composting. The concentration of Ag and Ti in mature samples was calculated using the following formula:

$$C = C_{in} \times (DW_{21} / DW_0)$$

where

C_{in} is the Ag or Ti concentration (mg/Kg) in biodegradable MSW,

C is the concentration (mg/kg) of Ag or Ti at mature compost samples,

DW_{21} is the dry weight (g) of the sample after 21 days of composting and

DW_0 is the dry weight (g) of the waste before composting.

The results are given in Table 3-14. Ten mature samples (one of each treatment) were acid digested and analysed using ICP-MS to determine the Ag and Ti concentrations. The results were very close to the average values given in Table 3-14. Low standard deviation values indicated the homogenous nature of the mature samples as well as the reproducibility of the analytical procedure.

Table 3-14. Ag and Ti concentration in mature samples.

Sample	Ag (mg/kg)		Ti (mg/kg)		sample	Ag (mg/kg)	
	Av	St Dev	Av	St Dev		Av	St Dev
AT 5	7.68	0.20	345.75	9.21	A 5	8.74	0.02
AT 10	16.39	0.41	737.54	18.61	A 10	19.39	0.16
AT 20	35.13	0.49	1580.75	21.98	A 20	36.06	1.60
AT 50	84.00	0.08	3779.81	3.68	A 50	84.39	4.15

3.2.3.2 Seed Germination Bioassays

The germination bioassays followed a modified procedure based on the one used by Zucconi *et al.* (1981) using cress seeds. Cress *(Lepidium Sativum)* seeds were purchased in sealed bags. Briefly, 3,5 gr (dw) of mature compost was mixed with UPW in an Erlenmeyer flask at a mixing ratio 10:1 (water volume, in ml, to dry weight, in g) and the mixture was then shaked during 30min in an orbital shaker at room temperature. The slurry was then filtered under vacuum using 0.45-μm filters

(Millipore). Cress seeds were placed on a No 42 Whatman ashless filter paper inside a 90mm diameter sterilized disposable Petri dish. The number of seeds used for the experiments were 20, which allowed enough space for seed growth. 5mL of compost filtrate was added to each Petri dish. Petri dishes were incubated in dark for 3 days at constant temperature, 20 ± 2 ^{o}C. 1 mL UPW was added the second day to each petri dish to keep moisture level constant (and compensate evaporation). The root length was measured after 3 days of incubation. Control experiments were conducted in triplicates using UPW and NPs solutions at the following concentrations: 5, 10, 20, 30 and 100 mg Ag/L.

The formula proposed by Tam and Tiquia (1994) was used to evaluate the seed germination bioassay. Seed germination, root growth and Germination Index (GI) were calculated as follows:

$$(\%)\ Seed\ germination = \frac{number\ of\ seeds\ germinated\ in\ extract}{number\ of\ seeds\ germinated\ in\ control} \times 100$$

$$(\%)\ Seed\ growth = \frac{Mean\ root\ growth\ in\ the\ extract}{Mean\ root\ growth\ in\ the\ control} \times 100$$

$$Germination\ Index\ (GI) = \frac{(\%\ Seed\ germination\ ) \times (\%Root\ growth)}{100}$$

$$Control:\ Germination\ Index = 100$$

3.2.3.3 Leaching experiments

A column leaching experiment was designed to investigate the leachable concentrations of AgNPs and Ag-TiO_2NPs from compost (Fig 3-40, Fig 3-41). The glass columns (8mm inner diameter and 300mm long) were filled in with 8g of coarse sand (<795μm) and 8g of fine sand (<595μm). At top layer, the column was filled with approximately 1g (dw) compost. Mature compost samples were used for the experiments. The samples were grinded to pass a 2 mm sieve. The experiments conducted in triplicates for AgNPs (AT5, AT10, AT20 and AT50) and Ag-TiO_2-NPs (A5, A10, A20 and A50).

UPW was supplied to each column, using a peristaltic pump, at a rate of 1mL min^{-1}. Leachate effluents were collected using 100 mL volumetric cylinders placed below the columns. Five samples of the leachate effluent of 50 mL each, referred to from

now on as sample 1, 2, 3, 4 and 5, were collected every 50 min and turbidity measured. The pH, EC, UV-254 and zeta potential of the leachate were measured after filtration using 0.45-μm filters (Millipore). Organic matter was monitored using fluorescence spectroscopy. Ag and Ti concentrations were determined using ICP-MS.

3.2.3.3.1 UV-vis

UV absorption was measured at λ=254nm using a Helios UV Visible Spectrophotometer. Concentrated samples with intensity more than 1Å at 254nm were diluted as needed. Samples were filtered using 0.45 μm filters (Millipore). The same cuvette was used for all the measurements. UPW was used as a blank sample.

3.2.3.3.2 Turbidity

The turbidity of the samples was measured with a HACH Model 2100N turbidimeter. The instrument was calibrated before the measurement using Gelex Secondary Turbidity Standards. The turbidity of the leachate samples were measured before filtration.

3.2.3.3.3 pH and EC

The pH and EC was measured using a Hanna 991300 pH meter. The instrument was calibrated before measurement using pH 4 and pH 7 buffer solutions an EC standard solution.

3.2.3.3.4 Organic matter investigation

The organic matter was investigated using EEM fluorescence spectroscopy. EEM spectra were obtained on leachate after dilution to eliminate the IFE. A Perkin-Elmer LS 55 fluorescence spectrophotometer equipped with FLWinlab for data processing was used for sample analysis. The emission wavelength (Em) range fixed from 200 to 600nm, whereas the excitation (Ex) wavelength increased from 200 to 600 nm keeping a constant Δλ of 25 nm.

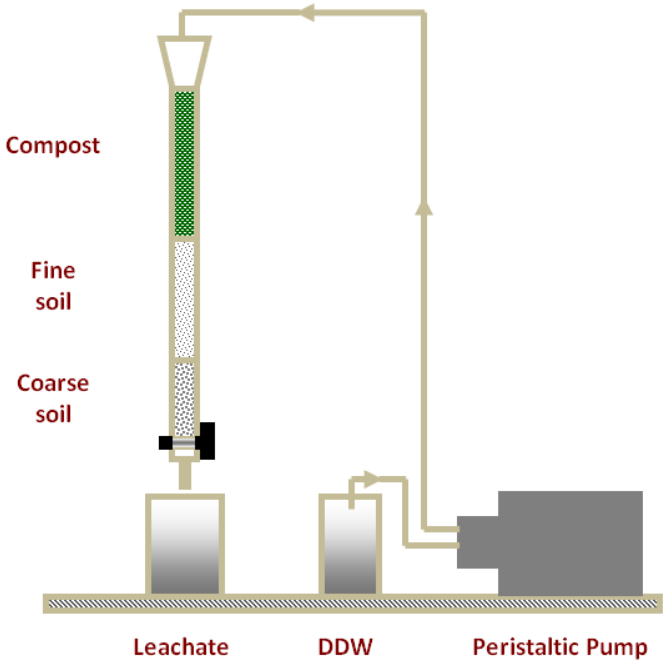

Fig 3-40. Leaching schematic set-up.

Fig 3-41. Leaching experimental set-up.

3.2.3.3.5 Ag and Ti concentrations

The concentrations of Ag and Ti in the samples were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). ICP-MS is applicable to the determination of sub-μg/L concentrations of a large number of elements in water samples and in waste extracts or digests (EPA 2007). According to the method, samples must be filtered and acid-preserved prior to the analysis of dissolved constituents but no digestion is required to analyse dissolved elements in water samples.

The compost samples were digested and analysed to determine the Ag and Ti concentration using ICP-MS. The USEPA Method 3052 Protocol was modified for the digestion of samples. This method is applicable to the microwave assisted acid digestion of siliceous matrices, and organic matrices and other complex matrices. The whole procedure is described in detail below:

1. A representative sample of 0.25g weight in a 100mL beaker, dried in oven at 105^{o}C for 24 h and the dry weight was calculated.
2. The sample was transferred to the furnace at 450^{o}C for 4 h and the ash content was calculated.
3. 10 mL of concentrated nitric acid (Aristar 68-70%) added to the sample, transferred in suitable inert polymeric vessels and digested using microwave heating for 15 min with a 14 vessel MARS microwave system. The temperature followed a ramp from room temperature 180^{o}C and then remained at 180^{o}C for 9 min.
4. After cooling during 30 min the vessels were emptied back to the beakers, rinsed with 2% nitric acid and placed on a hot plate to reduce the volume.
5. The sample was transferred to a volumetric flask, made up to 25mL using 2% nitric acid and filtered prior to analysis using No 40 Whatman filter paper.

To avoid cross-contamination, all digestion vessels were cleaned in a microwave using 10mL concentrated nitric acid and running a specific program for cleaning. Volumetric flasks and beakers were acid cleaned by boiling during 3 h in 30% nitric acid and boiling during 3 h in UPW prior to their use for the digestion process.

3.2.3.3.6 Atomic Force Microscopy

Atomic Force Microscopy was used to detect the size of AgNPs and Ag-TiO_2NPs in leachates. The leaching sample was filtered through 0.45 μm filter, applied to a mica sheet and left to evaporate overnight before analysing the surface. AFM imaging of the samples was carried out using a Bruker Multimode/ Nanoscope IIIa AFM (Bruker AXS, Santa Barbara, CA), equipped with an E-scanner (x-y scan range of *ca.* 10 microns). Bruker RTESP cantilevers were used, with nominal spring constant of 40 N/m, resonance frequency 300 kHz and tip curvature of *ca.* 8 nm, as specified by the manufacturer. The microscope was operated under tapping mode and all measurements were conducted in air at room temperature. AFM measurements were conducted at the Institute for Materials and Processes at the University of Edinburgh.

3.2.3.4 Quality Assurance and Quality Control

3.2.3.4.1 Seed Germination Bioassays

Sterilized Petri dishes were used for the experiments. The temperature was $20 \pm 2^{o}C$. A preliminary experiment in UPW was conducted to decide the optimum number of days for the experiment. The experiment ran for 6 days in triplicates with one triplicate for each day. The results for the mean root length are given in Table 3-15 and Figure 3-42. The results indicated that the optimum duration for the experiment was up to 3 days. After 3 days the standard deviation was considered high and thus it was not recommended to extent the experiment more than 3 days.

Table 3-15. Mean root growth (mm) for cress germination bioassays in UPW.

Mean root growth (mm)						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
1	4.20	20.05	39.20	60.95	59.95	58.70
2	3.95	20.55	39.25	50.65	59.55	85.75
3	4.15	18.85	38.55	55.65	67.45	72.85
Av	**4.10**	**19.82**	**39.00**	**55.75**	**62.32**	**72.43**
St Dev	0.13	0.87	0.39	5.15	4.45	13.53

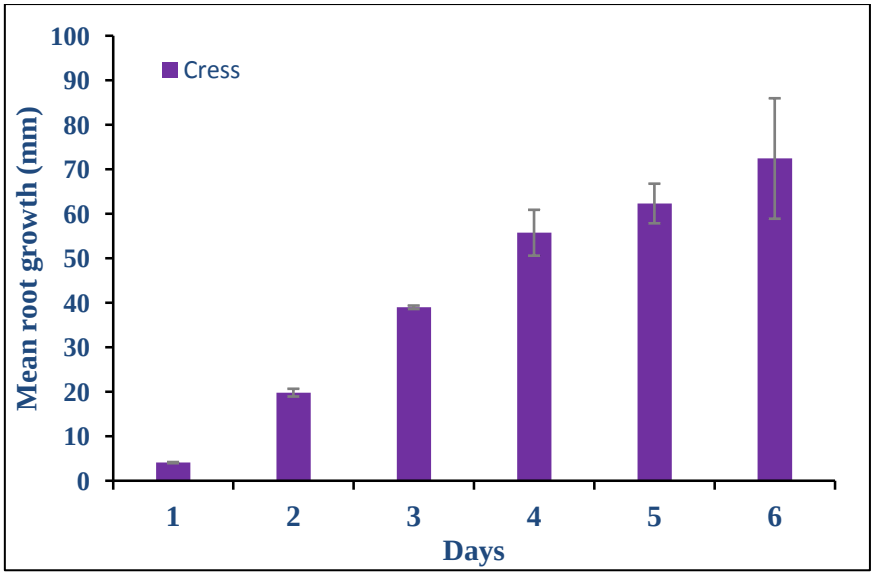

Fig 3-42. Mean root growth (mm) for cress germination bioassays in UPW.

3.2.3.4.2 Leaching tests

A control leaching experiment using non-contaminated compost was conducted and the concentrations of Ag and TiO_2 in leachate detected using ICP-MS. The concentrations were the same as blank samples used in ICP-MS.

Another control leaching experiment was conducted by filling the column only with sand and pH, EC, turbidity and UV absorption at λ=254nm were measured. Ag and TiO_2 concentrations were monitored using ICP-MS and were the same as the blank samples used for the analysis. Also, OM was monitored using fluorescence spectroscopy. The EEM spectra is presented in Figure 3-42.

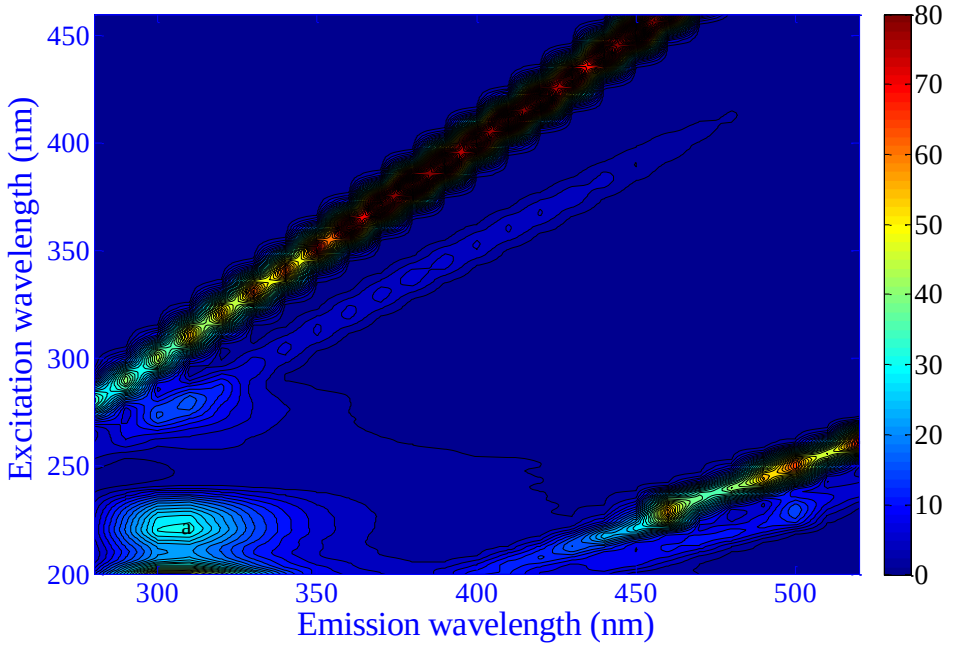

Fig 3-43. EEM contour map for sample 5 of sand control experiment.

3.2.4 Results and Discussion

3.2.4.1 Seed Germination

The germination index (GI) was calculated according to the formula given in 3.2.3.2 sub-chapter. The results of the experiments conducted using Ag-TiO_2NPs-contaminated compost samples are presented in Fig 3-44 (see also Appendix B). In general, all the compost samples, both contaminated and non-contaminated, exhibit higher GI compared to the GI of the control (UPW). This may be attributed to the nutrients supplied from compost such as N, P and K. The experiment AT 5 exhibit the highest GI and the AT 50 the lowest but very close to values recorded in experiments AT 0 and AT 10 and AT 20.

The concentration of Ag in compost was measured in order to estimate the range of Ag concentrations the seeds would be exposed to. The highest Ag concentration expected in compost extracts was lower than 5 mg Ag / L when using AT 50 so the concentration values of compost extracts ranged from 0-5 mg Ag / L for all treatments investigated. The concentration was calculated assuming that all the amount of Ag present in the samples could be released from the mature compost to the extract.

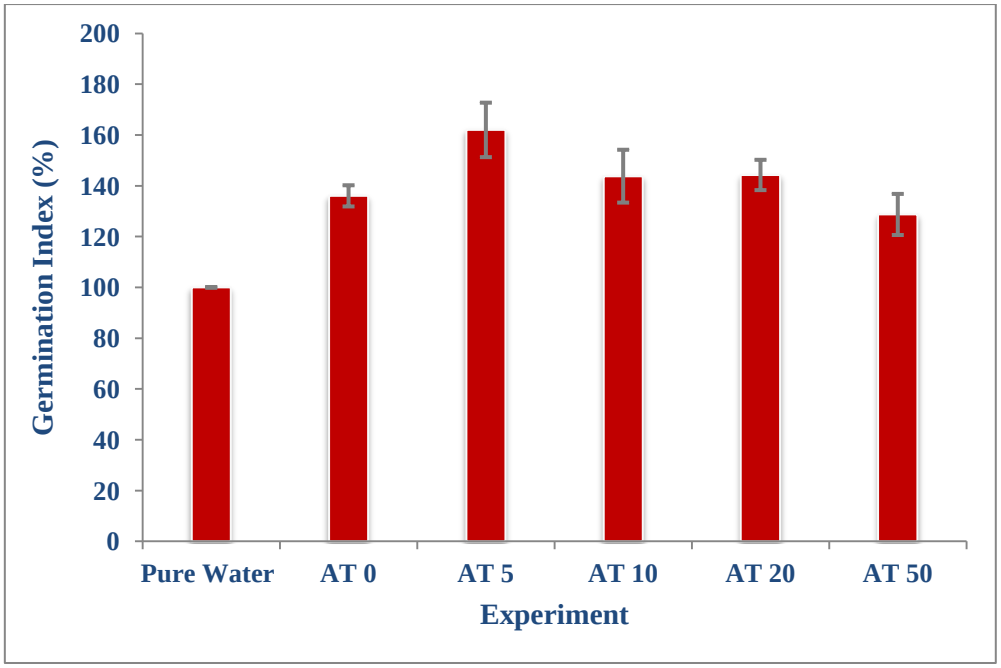

Fig 3-44. Germination Index (%) for the experiments conducted using compost samples contaminated with Ag-TiO_2-NPs.

The GI of the experiments conducted using Ag-TiO_2NPs solutions are presented in Figure 3-45 (see also Appendix B). The concentration of 5 mg Ag /L exhibit similar GI with the GI of the control (UPW). The GI of the seed exposed to higher concentrations of Ag decreased as Ag concentration increased, and was as low as 35% for the concentration of 100 mg Ag / L. In the range of 0-5 mg Ag / L no toxic effects were observed.

The results indicated that the GI was highest for AT 5 and then decreased as the concentration of Ag and TiO_2 increases. This may imply that Ag or Ti presence had an effect on the availability of nutrients to the seeds as the NPs did not exhibit any toxicity at the range of concentrations that seeds were exposed to.

The results for the experiments conducted using compost samples contaminated with AgNPs are presented in Figure 3-46 (see also Appendix B). In general, all the compost samples, both contaminated and non-contaminated, exhibited higher GI compared to the GI of the control (UPW). This can be attributed to the nutrients supplied from compost such as N, P and K. The experiment A 10 exhibited the highest GI. The experiments A 0, A 20 and A 50 exhibited similar GI whereas the GI of A 5 was slightly higher.

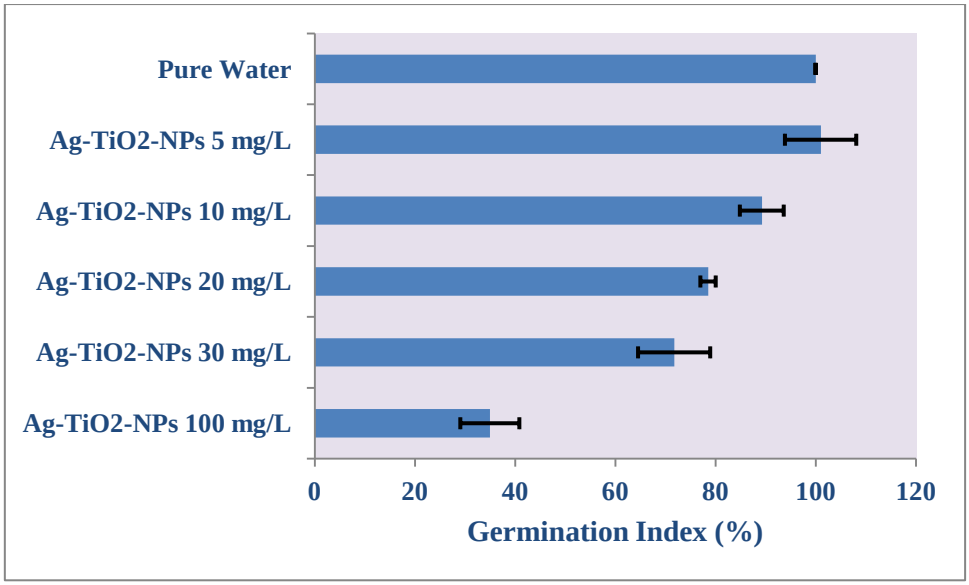

Fig 3-45. Germination Index (%) for the experiments conducted using pure solution of Ag-TiO_2-NPs.

The GI of the experiments conducted using AgNPs solutions are presented in Figure 3-47 (see also Appendix B). The experiments using solutions with concentrations of 20, 30 and 50 mg Ag / L exhibited lower GI as compared to the GI of the control (UPW). In the range of 0-5 mg Ag / L no toxic effects were observed. According to these results there was no expectation of toxicity due to the presence of AgNPs at the range of concentration that seeds were exposed to. The difference may be attributed to the higher HA and ammonia concentration of A 5 and A10 as compared to the other treatments investigated here.

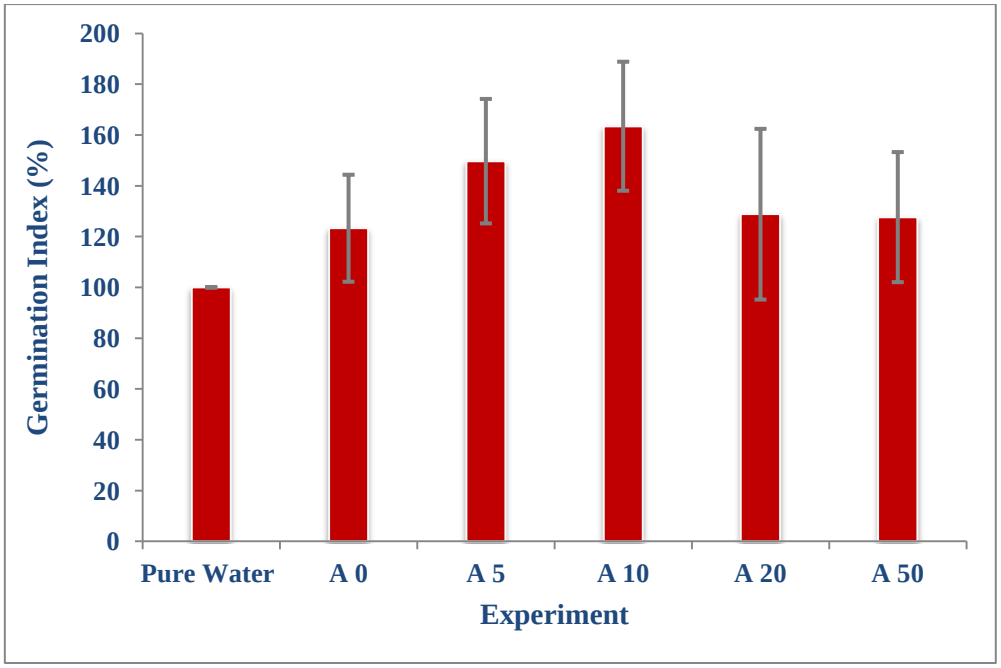

Fig 3-46. Germination Index (%) for the experiments conducted using compost samples contaminated with AgNPs.

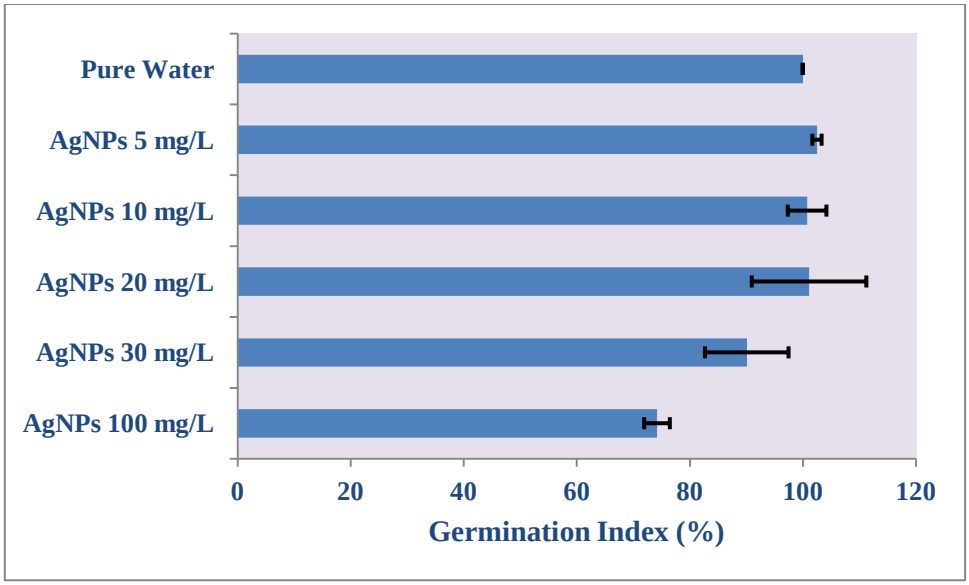

Fig 3-47. Germination Index (%) for the experiments conducted using pure solution of AgNPs.

These results indicated that NP-contaminated compost presented no phytotoxic effect to the cress seed germination even at high concentrations of AgNPs and Ag-TiO_2NPs in compost. Although the toxicity of Ag ionic form is well-understood, the toxicological impact of AgNPs is more complicated due largely to uncertainties in the amounts of aggregation or exposure (Mirzajani, Askari et al. 2013). A number of factors related with plants (species, seeds etc.), AgNPs (size, concentration, coatings,

aggregation), environmental/experimental conditions (temperature, time, method of exposure) may control AgNP-phytotoxicity (Navarro, Baun et al. 2008). There is evidence from several reports that AgNPs interactions with soils and plants have a negative effect but the potential mechanisms underlining AgNP-uptake and AgNP-phytotoxicity remain largely unknown (Anjum, Gill et al. 2013).

3.2.4.2 Leaching Experiments

3.2.4.2.1 Silver doped titanium dioxide experiments

The pH values of the leaching samples ranged between 7.03 and 8.96. The actual values are given in Table 3-16, 3-17 and Figure 3-48. The values are the average of triplicate analysis. Experiment AT 50 resulted in higher pH values as compared to the other composts samples. The pH values of experiments AT 5, AT 10 and AT 20 were in the neutral region for the first leaching sample and then decreased to slightly acidic values for the rest of the leaching samples.

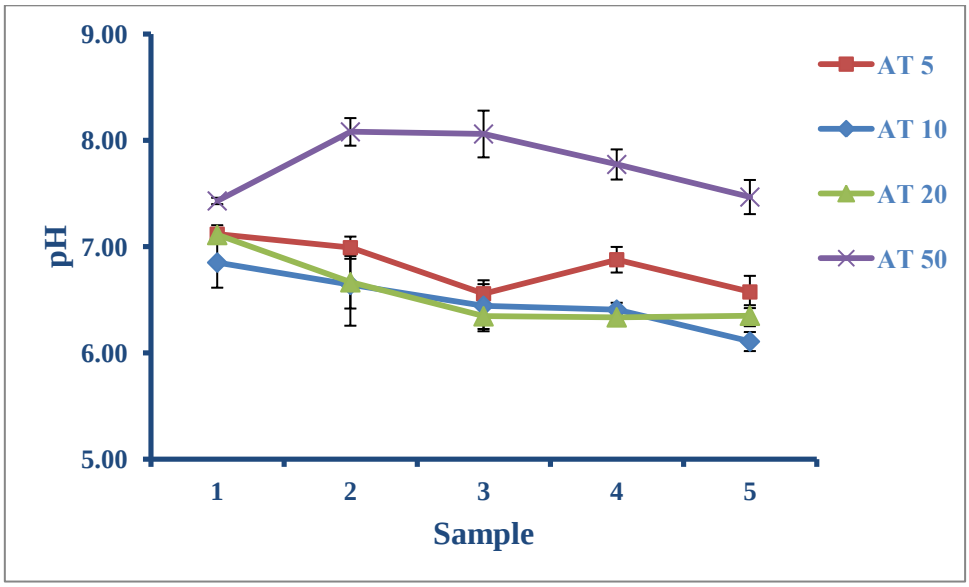

Fig 3-48. The pH of the leaching samples for different concentrations of Ag-TiO_2NPs in compost.

The turbidity values were higher for the first leaching sample and then decreased for all the rest of the leaching samples (see Tables 3-16, 3-17 and Figure 3-49). Higher turbidity values resulted for NP-contaminated compost with a higher concentration of Ag-TiO_2NPs.

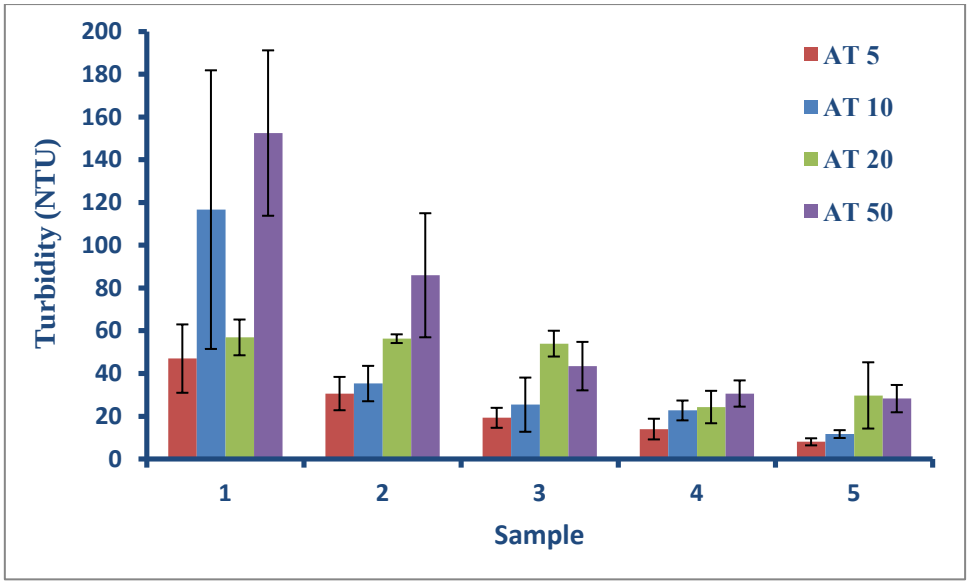

Fig 3-49. The turbidity of the leaching samples for different concentrations of Ag-TiO_2NPs in compost.

The UV absorption values of the leaching samples at λ=254nm were relatively higher for the first collected sample and then subsequently decreased for the rest of the samples; this was observed for all the experiments conducted at different concentrations of NPs (see Tables 3-16, 3-17 and Figure 3-50).

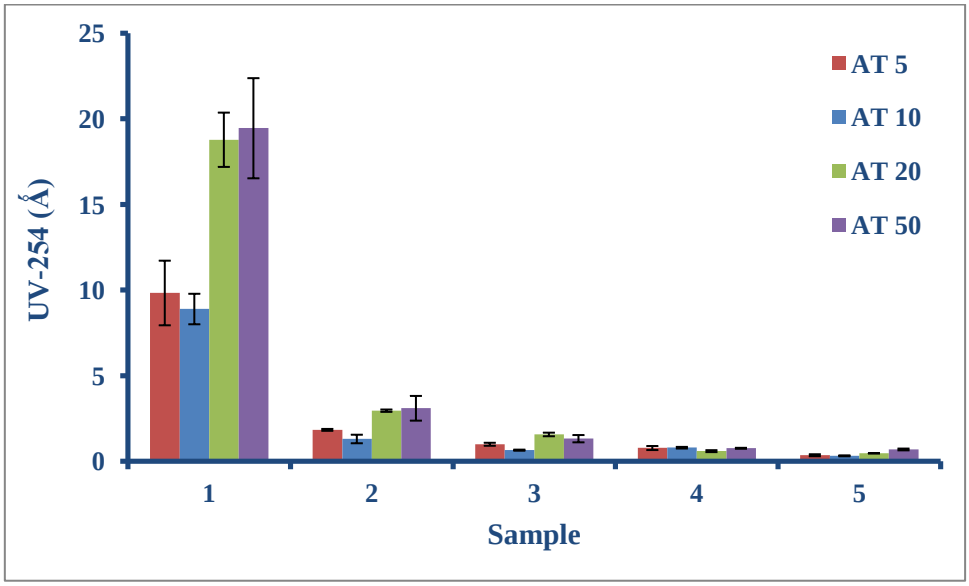

Fig 3-50. The UV absorption values at λ=254 nm of the leaching samples for different concentrations of Ag-TiO_2NPs in compost.

The zeta potential values are given in Table 3-16, 3-17 and Figure 3-51. Apart of the first leaching sample, the samples exhibited very good stability as the values were

close to -30mV. This may be attributed to the presence of HS that can stabilise the NPs in solution. AT 50 showed large standard deviations for samples 2-5 and low stability for sample 1. The explanation may be that the concentration of Ag-TiO_2NPs was higher than the binding capacity of HS. It has been suggested that any high-affinity binding sites present within the HA are likely saturated at Ag concentrations higher than 10^{-9} M (Chen, Campbell et al. 2012).

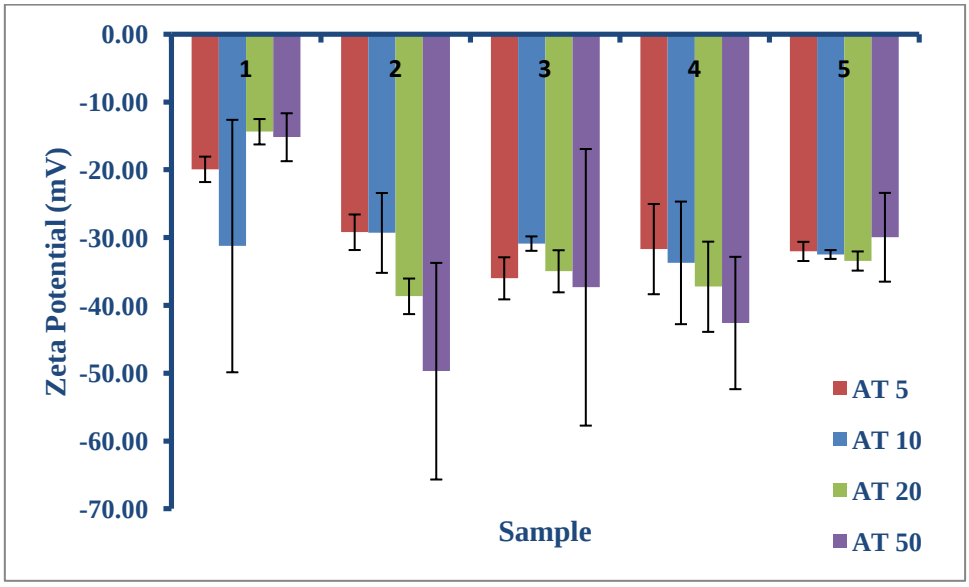

Fig 3-51. The zeta potential of the leaching samples for different concentrations of Ag-TiO_2NPs in compost.

The concentrations of Ag and Ti were higher for the first leaching sample and then decreased for all the rest of the samples (see Tables 3-16, 3-17 and Figure 3-52). Experiment AT 50 exhibited high standard deviations that may be attributed to the high NPs concentration. High concentrations of Ag and Ti were found in the first leaching sample as compared to the rest of the samples.

A qualitative analysis was conducted using EEM fluorescence spectroscopy to characterise the OM of the leaching samples. The samples were diluted to obtain a value of 0.3 Å when measure UV absorbance at λ=254 nm. The EEM contour maps for experiments AT 5, AT 10, AT 20 and AT 50 are presented in Figures 3-53, 3-54, 3-55 and 3-56 respectively.

The leaching sample 1 resulted in higher concentration of HS for all the experiments. A peak in the area of AP I appeared in leaching sample 5 for all the experiments. This peak can be attributed to: (a) the sand used for the experiments (see Figure 3-43) and/or (b) the OM that have not totally decomposed and detected in FA fraction (see

Figure 3-22). The peak appeared due to the low concentration of the HS which allow the detection of the peak without any overlapping with the HS peaks. Also peaks in the area of AP II and SM appeared and can be attributed to the OM that have not totally decomposed and detected in FA fraction.

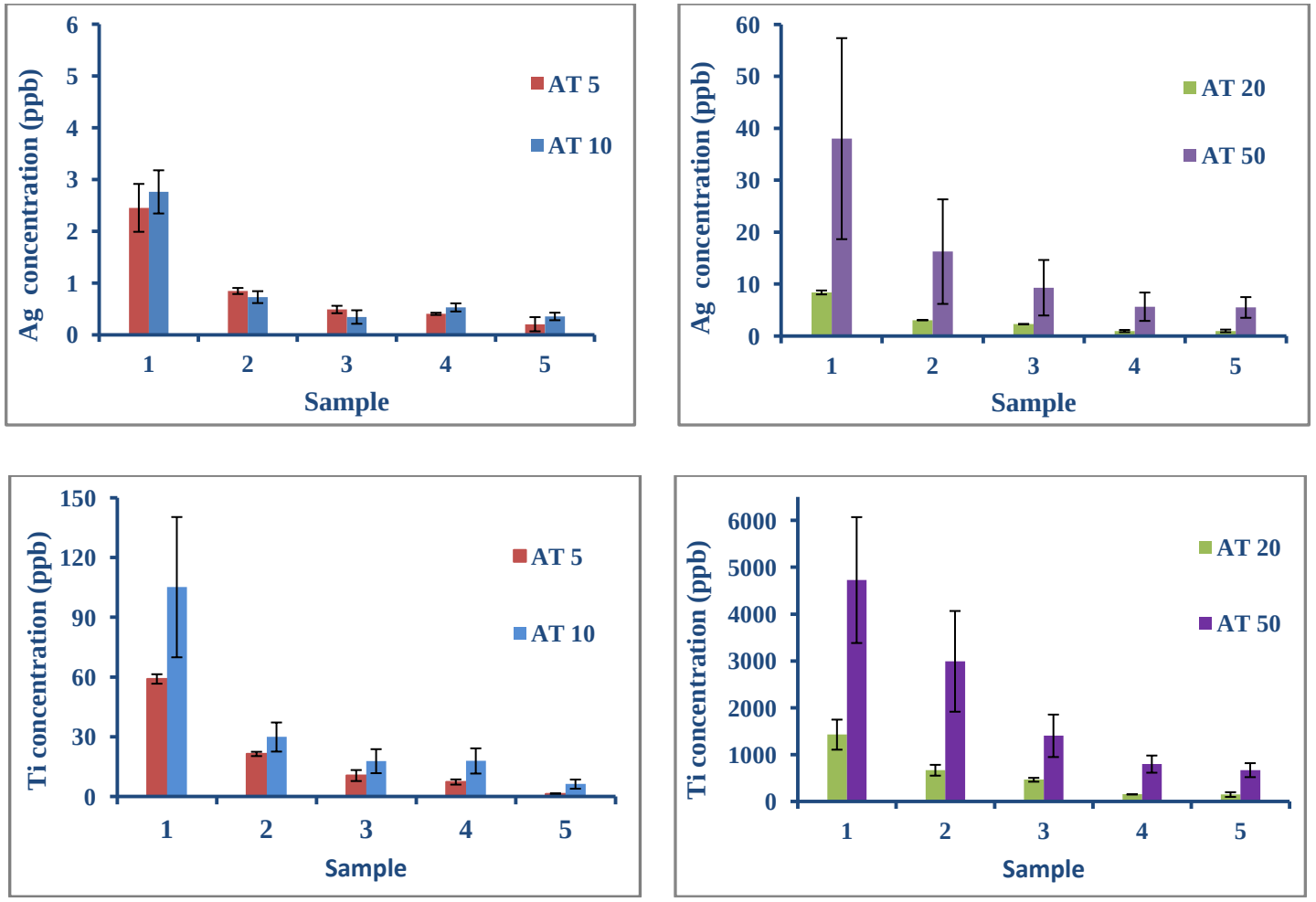

Fig 3-52. The concentration of Ag and Ti in leaching samples for different concentrations of of Ag-TiO_2NP's in compost.

Table 3-16. The values of pH, EC, turbidity, UV-254, zeta potential and concentrations of Ag and Ti in leaching samples for AT 5 and AT 10. The values are the average of triplicate analysis plus standard deviation.

AT 5													
Leaching sample	**pH**		**EC (µS/cm)**		**Turbidity (NTU)**		**UV-254 (Å)**		**Zeta Potential**		**Ag (µg/L)**		**Ti (µg/L)**	
	Av	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**
1	7.12	0.09	799.86	17.88	46.97	16.00	9.832	1.887	-19.92	5.416	2.452	0.463	59.084	2.370
2	6.99	0.10	83.14	42.30	30.61	7.79	1.837	0.055	-29.197	2.402	0.846	0.059	21.431	1.073
3	6.56	0.09	45.38	7.98	19.27	4.67	0.996	0.088	-35.993	4.869	0.488	0.072	10.574	2.759
4	6.88	0.12	30.49	1.38	14.00	4.84	0.777	0.117	-31.683	2.971	0.404	0.022	7.334	1.301
5	6.57	0.15	25.52	5.02	8.03	1.65	0.354	0.059	-32.023	1.661	0.204	0.137	1.353	0.334
AT 10														
Leaching sample	**pH**		**EC (µS/cm)**		**Turbidity (NTU)**		**UV-254 (Å)**		**Zeta Potential**		**Ag (µg/L)**		**Ti (µg/L)**	
	Av	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**
1	6.85	0.24	927.70	22.11	116.66	65.18	8.89	0.89	-31.23	18.613	2.76	0.417	105.19	35.223
2	6.64	0.38	61.67	18.66	35.30	8.27	1.30	0.25	-29.297	5.889	0.727	0.115	29.921	7.306
3	6.44	0.24	34.27	3.10	25.41	12.68	0.65	0.03	-30.873	1.064	0.343	0.130	17.792	6.023
4	6.41	0.07	37.72	6.91	22.72	4.64	0.80	0.05	-33.713	9.039	0.528	0.079	17.908	6.326
5	6.11	0.09	24.55	3.16	11.66	1.83	0.32	0.03	-32.480	0.650	0.354	0.073	6.273	2.299

Table 3-17. The values of pH, EC, turbidity, UV-254, zeta potential and concentrations of Ag and Ti in leaching samples for AT 20 and AT 50. The values are the average of triplicate analysis plus standard deviation.

AT 20														
Leaching sample	**pH**		**EC (µS/cm)**		**Turbidity (NTU)**		**UV-254 (Å)**		**Zeta Potential**		**Ag (µg/L)**		**Ti (µg/L)**	
	Av	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**
1	7.11	0.06	1070.36	33.34	56.89	8.37	18.78	1.59	-14.38	1.87	8.393	0.380	1430.60	320.61
2	6.67	0.25	81.00	2.08	56.29	2.04	2.96	0.07	-38.65	2.62	3.068	0.008	670.93	115.38
3	6.35	0.12	50.92	0.89	53.98	6.03	1.57	0.10	-34.96	3.10	2.290	0.017	467.90	41.10
4	6.34	0.02	34.75	3.79	24.31	7.58	0.59	0.06	-37.24	6.65	0.961	0.212	156.23	2.45
5	6.35	0.10	36.58	16.09	29.74	15.50	0.47	0.02	-33.44	1.41	0.986	0.258	152.11	50.20
AT 50														
Leaching sample	**pH**		**EC (µS/cm)**		**Turbidity (NTU)**		**UV-254 (Å)**		**Zeta Potential**		**Ag (µg/L)**		**Ti (µg/L)**	
	Av	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**	**Av**	**StDev**
1	7.43	0.03	1155.53	102.67	152.50	38.69	19.456	2.925	-15.18	3.53	37.995	19.349	4728.79	1342.85
2	8.08	0.13	107.75	35.28	85.97	29.02	3.098	0.721	-49.69	15.97	16.250	10.066	2994.48	1074.58
3	8.06	0.22	51.29	12.23	43.46	11.34	1.322	0.213	-37.32	20.39	9.301	5.341	1405.97	452.80
4	7.77	0.14	31.92	1.57	30.63	6.14	0.761	0.027	-42.58	9.76	5.647	2.729	801.49	183.20
5	7.47	0.16	28.25	6.39	28.25	6.39	0.693	0.052	-29.94	6.55	5.501	1.995	673.22	149.22

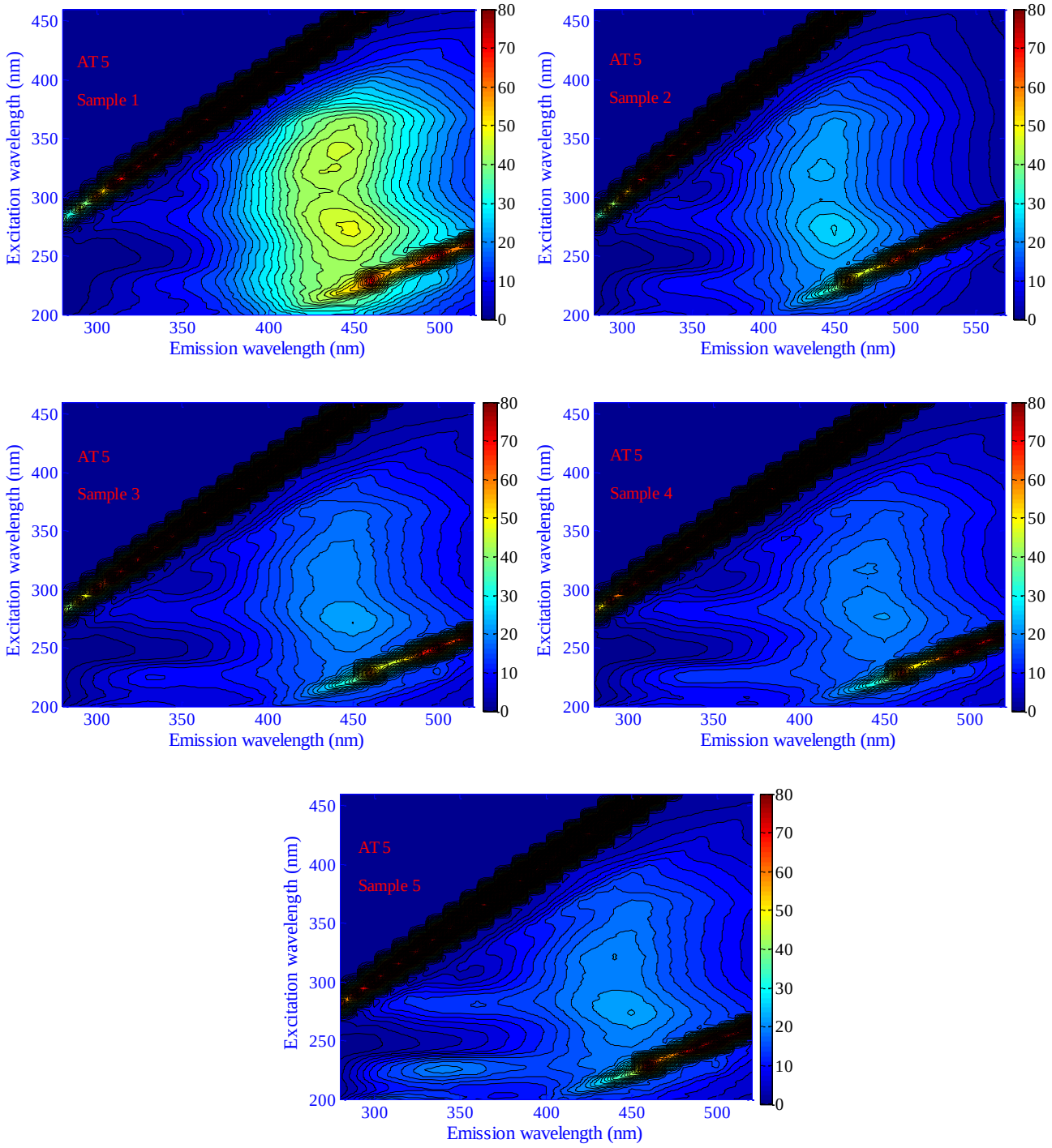

Fig 3-53. EEM contour maps for AT 5 during leaching tests.

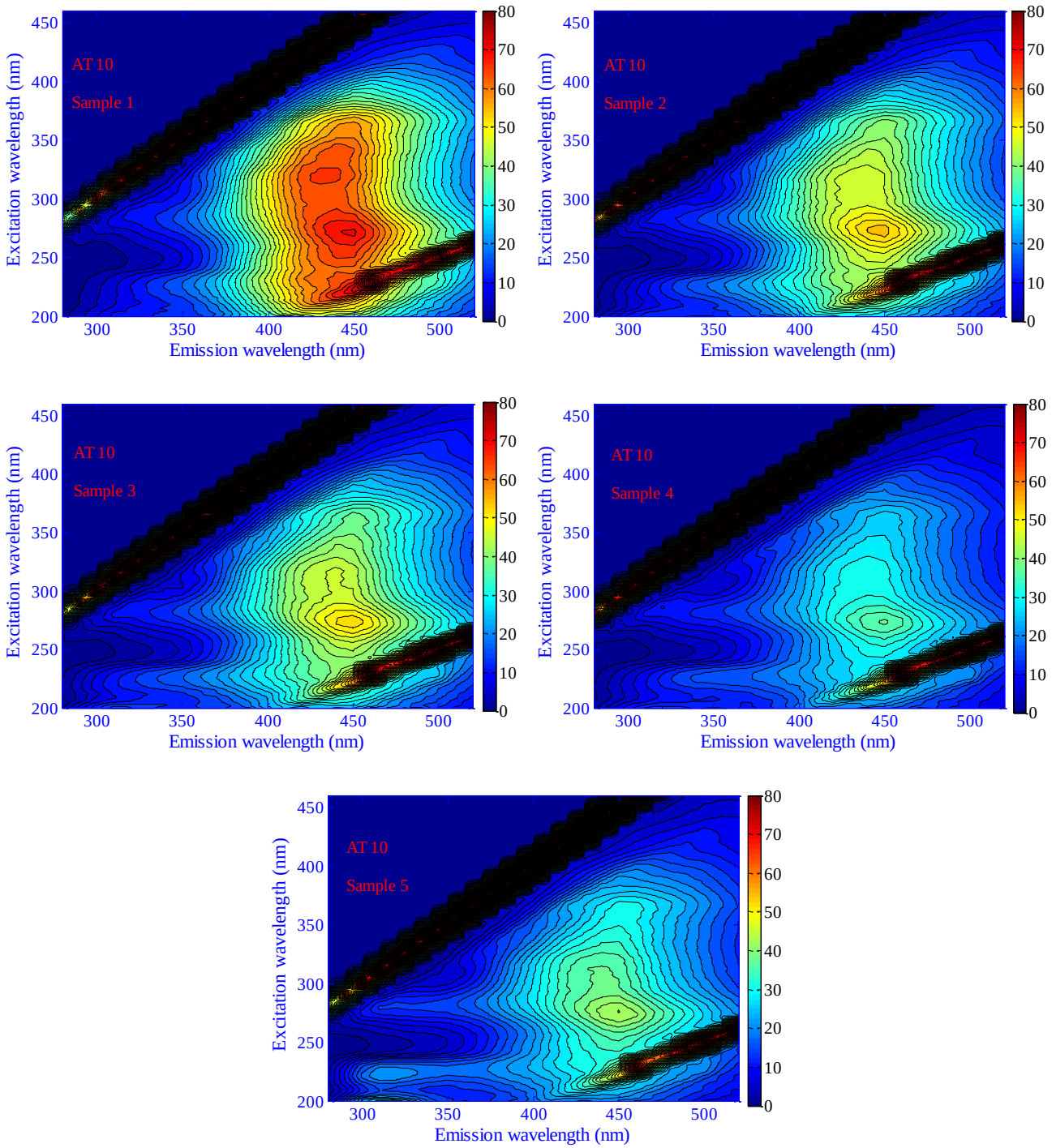

Fig 3-54. EEM contour maps for AT 10 during leaching tests.

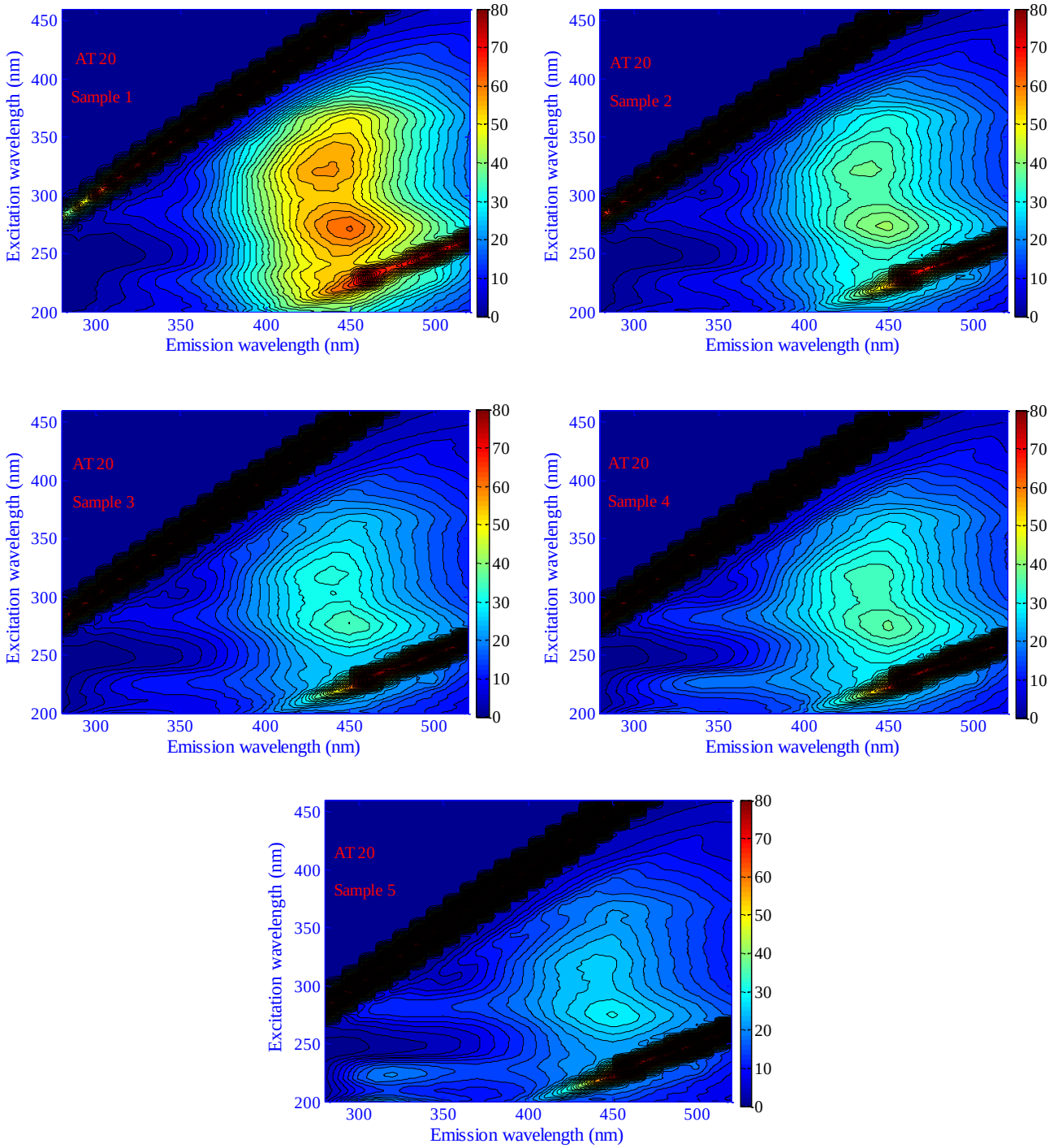

Fig 3-55. EEM contour maps for AT 20 during leaching tests.

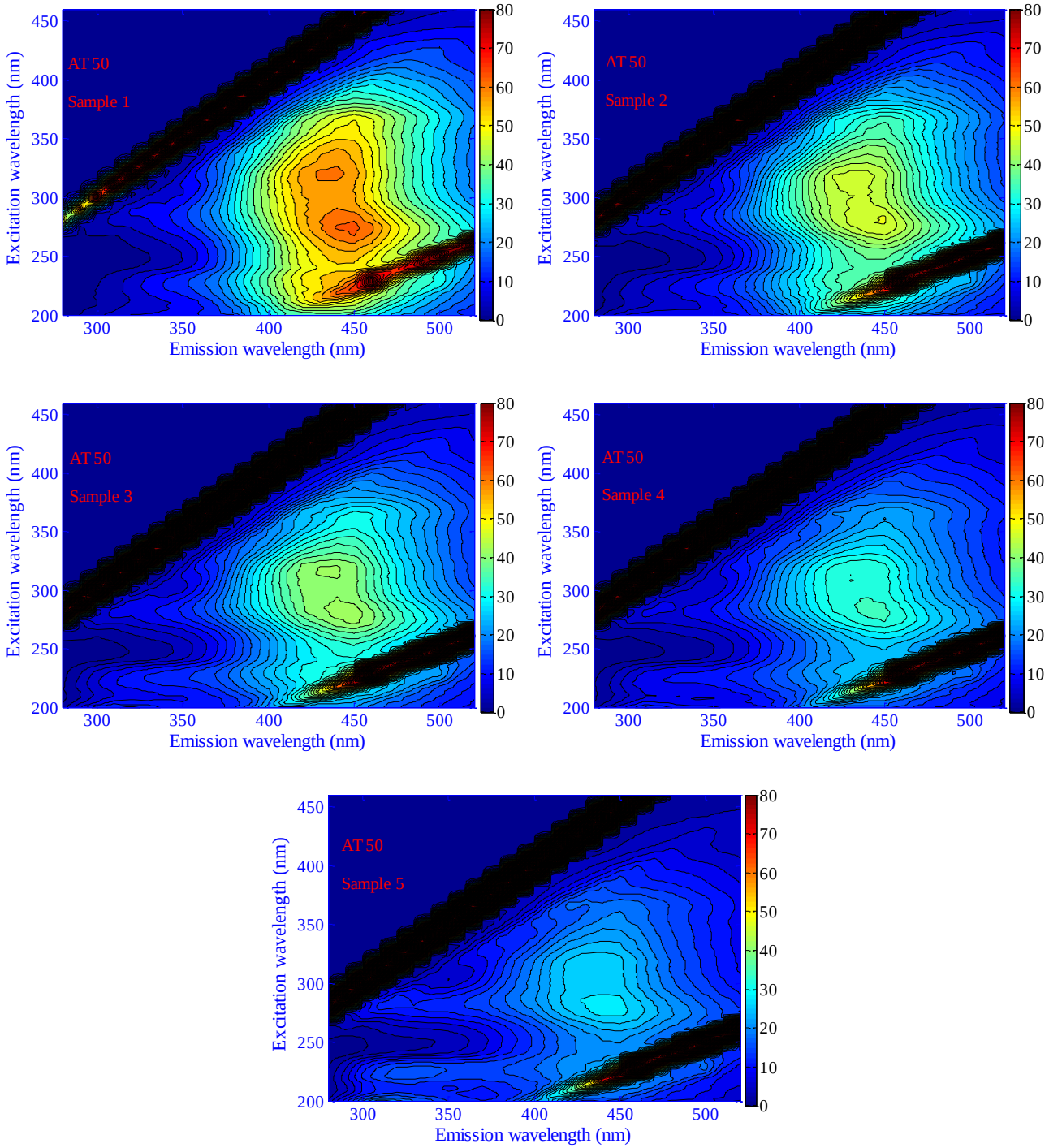

Fig 3-56. EEM contour maps for AT 50 during leaching tests.

The total mass (µg) and the percentage of Ag and Ti present in DOM leached from the compost samples during the leaching experiment were calculated. The results are given in Table 3-18 and Table 3-19.

Table 3-18. The total mass in µg of Ag and Ti determined in the five leaching samples.

Sample	Ag (µg)		Ti (µg)	
	Av	St Dev	Av	St Dev
AT 5	0.205	0.001	5.075	0.082
AT 10	0.236	0.024	8.775	0.867
AT 20	0.785	0.017	143.88	26.242
AT 50	3.735	1.953	530.98	154.51

Table 3-19. The percentage of Ag and Ti leached from the compost samples.

Sample	% Ag	% Ti
AT 5	2.679	1.468
AT 10	1.438	1.190
AT 20	2.234	9.105
AT 50	4.447	14.028

The particle size of NPs was estimated for leaching sample 3 of AT 20 in order to elucidate whether aggregation would occur. The sample was analysed using atomic force microscopy. The results are presented in Figure 3-57. The particle size falls in the nano-region (1-100nm). The detected peaks were between 5-25 nm. This may indicate that no aggregation occurred after leaching of the NP-contaminated compost to the environment. This result is in agreement with what was expected due to the presence of HS that act as a stabilizer for the NPs in the solution.

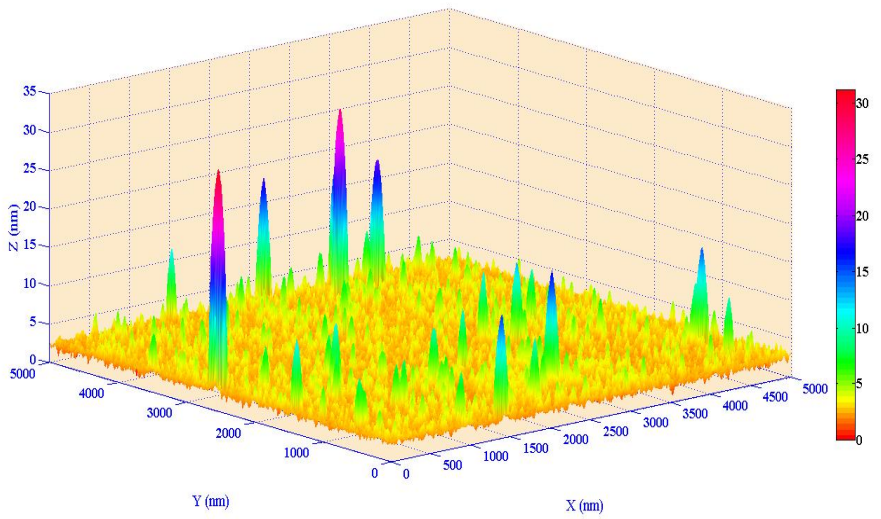

Fig 3-57. Image from atomic force microscopy to detect the particle size of the Ag-TiO_2-NPs.

3.2.4.2.2 AgNPs experiments

The pH values of the leaching samples ranged between 5.41 and 6.96. The values are given in Tables 3-20, 3-21 and Figure 3-58. The values are the average of triplicate analysis. The pH values were higher and almost neutral for the first leaching sample and then decreased to slightly acidic values for the rest of the leaching samples.

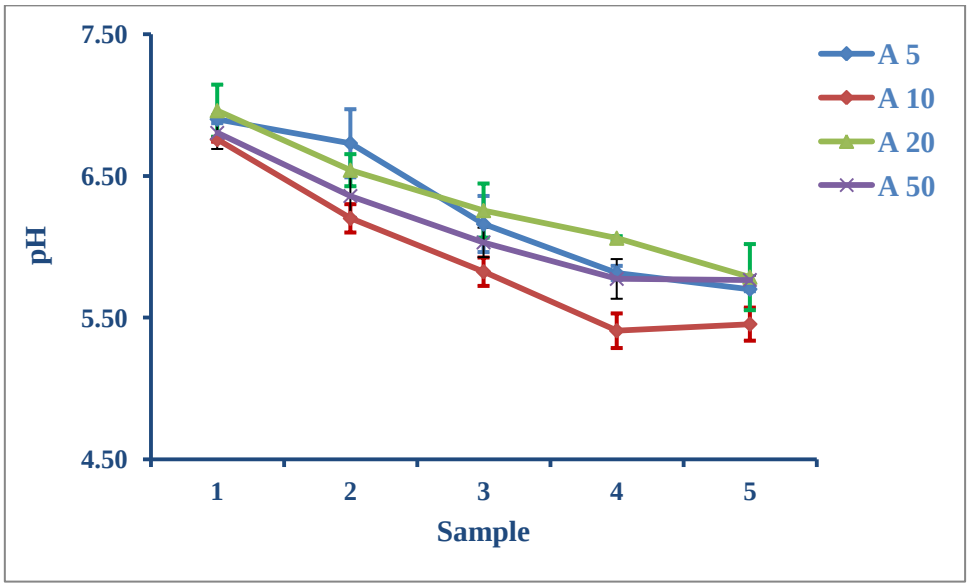

Fig 3-58. The pH of the leaching samples for different concentrations of AgNPs in compost.

The turbidity values were higher for the first leaching sample and then decreased for all the experiments conducted (see Tables 3-20, 3-21 and Figure 3-59).

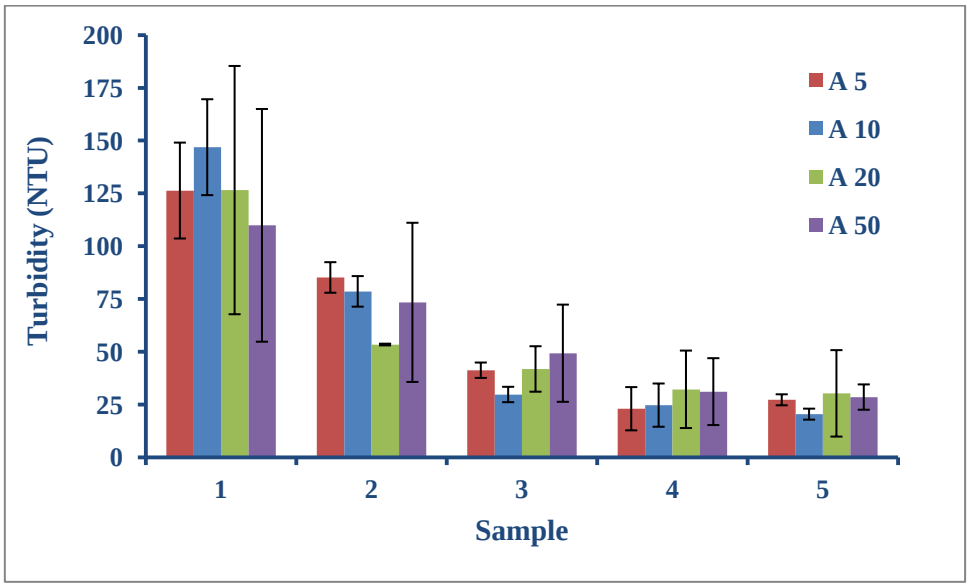

Fig 3-59. The turbidity of the leaching samples for different concentrations of AgNP's in compost.

The UV absorption values of the leaching samples at λ=254nm were much higher for the first sample and then reduced for the rest of the samples (see Tables 3-20, 3-21 and Figure 3-60).

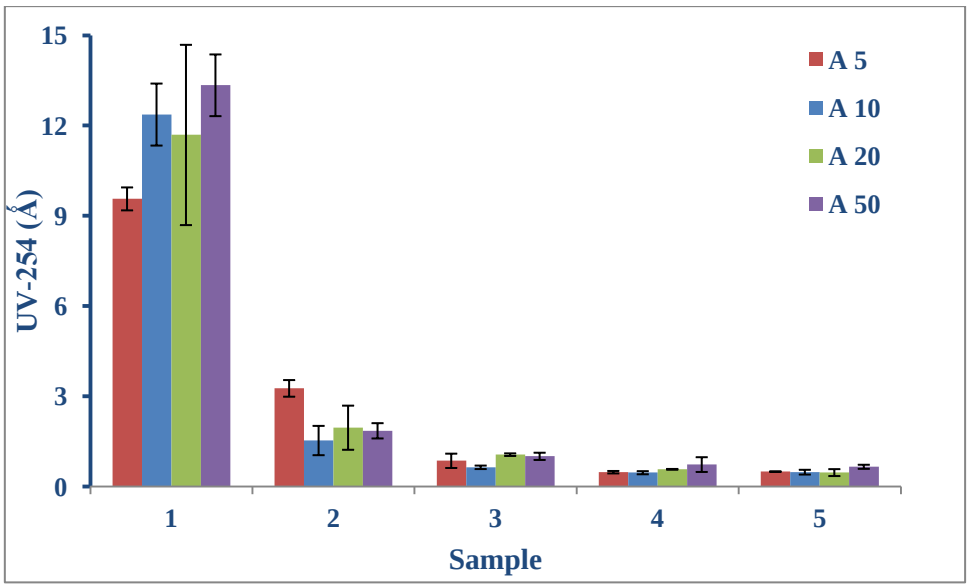

Fig 3-60. The UV absorbance values at λ=254nm of the leaching samples for different concentrations of AgNP's in compost.

Higher Ag concentrations were detected for the first leaching samples (see Tables 3-20, 3-21 and Figures 3-61, 3-62). The leaching sample 1 resulted in the highest Ag concentration for all the experiments conducted.

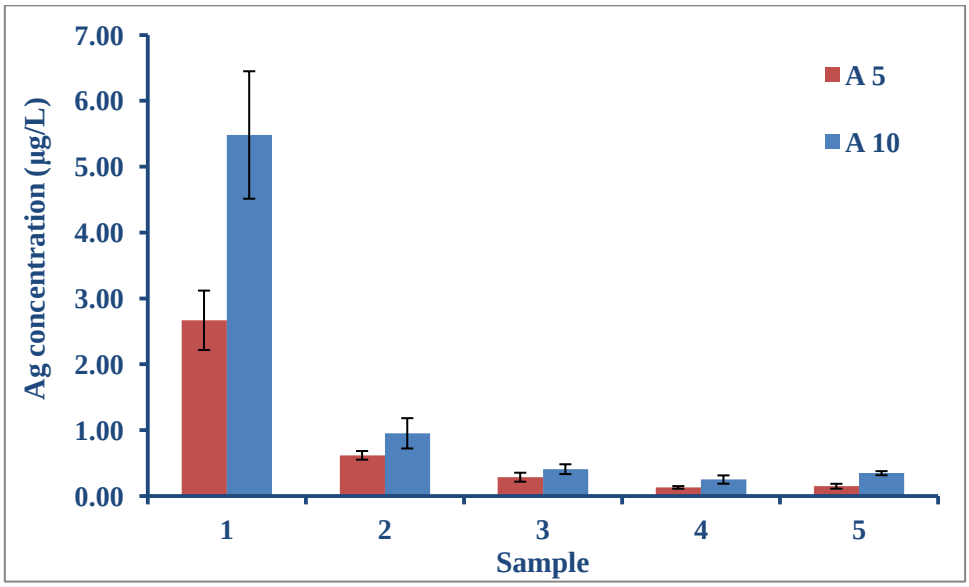

Fig 3-61. Ag concentration in µg/L of the leaching samples for A 5 and A 10.

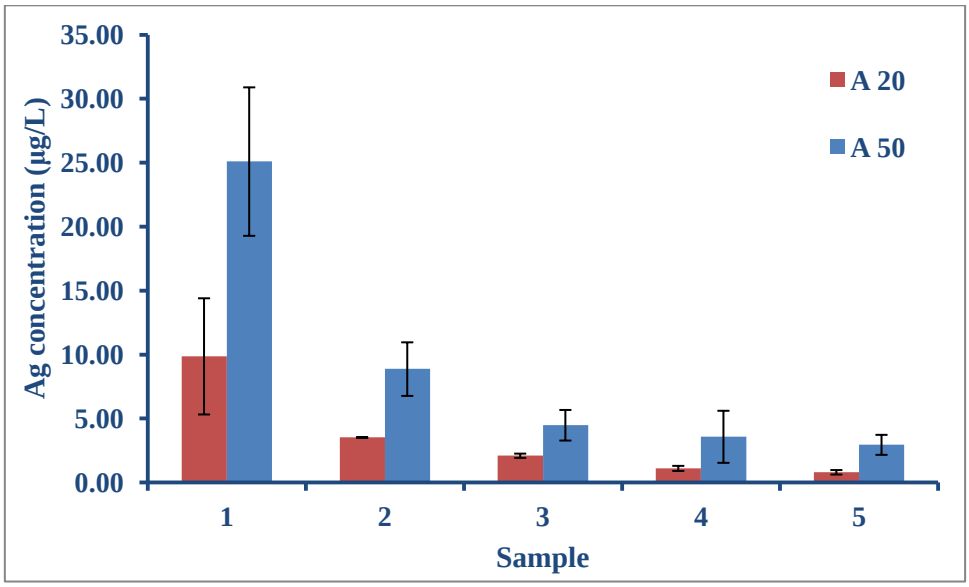

Fig 3-62. Ag concentration in µg/L of the leaching samples for A 20 and A 50.

A qualitative analysis was conducted using EEM fluorescence spectroscopy to determine the OM of the leaching samples. The samples were diluted to obtain a value of 0.3 Å when measure UV absorbance at λ=254 nm. The EEM contour maps for experiments A 5, A 10, A 20 and A 50 are presented in Figures 3-63, 3-64, 3-65 and 3-66 respectively.

Table 3-20. The values of pH, EC, turbidity, UV-254 and Ag concentration in leaching samples for A 5 and A10. The values are the average of triplicate analysis plus standard deviation.

A 5										
Leaching sample	**pH**		**EC (µS/cm)**		**Turbidity (NTU)**		**UV-254 (Å)**		**Ag (µg/L)**	
	Av	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**
1	6.90	0.03	1155.15	54.65	126.29	74.04	9.566	0.38	2.67	0.451
2	6.73	0.24	115.47	67.37	85.12	14.31	3.26	0.28	0.618	0.066
3	6.16	0.20	36.86	5.54	41.18	6.02	0.85	0.24	0.287	0.068
4	5.82	0.05	21.33	5.46	22.94	6.82	0.48	0.04	0.132	0.020
5	5.70	0.01	24.24	6.83	27.16	8.82	0.49	0.01	0.150	0.037
A 10										
Leaching sample	**pH**		**EC (µS/cm)**		**Turbidity (NTU)**		**UV-254 (Å)**		**Ag (µg/L)**	
	Av	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**
1	6.76	0.02	1153.35	85.43	146.84	22.72	12.370	1.031	5.482	0.968
2	6.20	0.10	61.28	19.40	78.53	7.24	1.529	0.487	0.953	0.230
3	5.82	0.10	26.42	1.63	29.68	3.65	0.638	0.058	0.409	0.075
4	5.41	0.12	18.27	3.27	24.65	10.24	0.458	0.052	0.251	0.063
5	5.45	0.12	23.45	6.74	20.37	2.61	0.476	0.079	0.348	0.031

Table 3-21. The values of pH, EC, turbidity, UV-254 and Ag concentration in leaching samples for A 20 and A 50. The values are the average of triplicate analysis plus standard deviation.

A 20										
Leaching sample	**pH**		**EC (µS/cm)**		**Turbidity (NTU)**		**UV-254 (Å)**		**Ag (µg/L)**	
	Av	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**
1	6.96	0.18	1259.11	160.49	126.51	58.81	11.692	2.998	9.873	4.541
2	6.54	0.11	118.76	72.56	53.35	0.41	1.955	0.735	3.531	0.017
3	6.26	0.19	54.67	7.66	41.77	10.77	1.059	0.043	2.102	0.168
4	6.06	0.01	25.60	1.65	32.14	18.35	0.571	0.014	1.116	0.198
5	5.79	0.23	18.29	1.39	30.22	20.48	0.462	0.115	0.810	0.175
A 50										
Leaching sample	**pH**		**EC (µS/cm)**		**Turbidity (NTU)**		**UV-254 (Å)**		**Ag (µg/L)**	
	Av	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**	**Av**	**St Dev**
1	6.80	0.11	1067.21	38.08	109.82	55.12	13.344	1.03	25.10	5.804
2	6.36	0.15	91.33	36.55	73.33	37.70	1.85	0.25	8.877	2.094
3	6.03	0.10	36.89	5.37	49.24	23.02	1.00	0.12	4.487	1.196
4	5.77	0.14	28.98	9.63	31.05	15.84	0.73	0.25	3.584	2.035
5	5.77	0.03	26.04	2.47	28.46	6.00	0.66	0.07	2.956	0.782

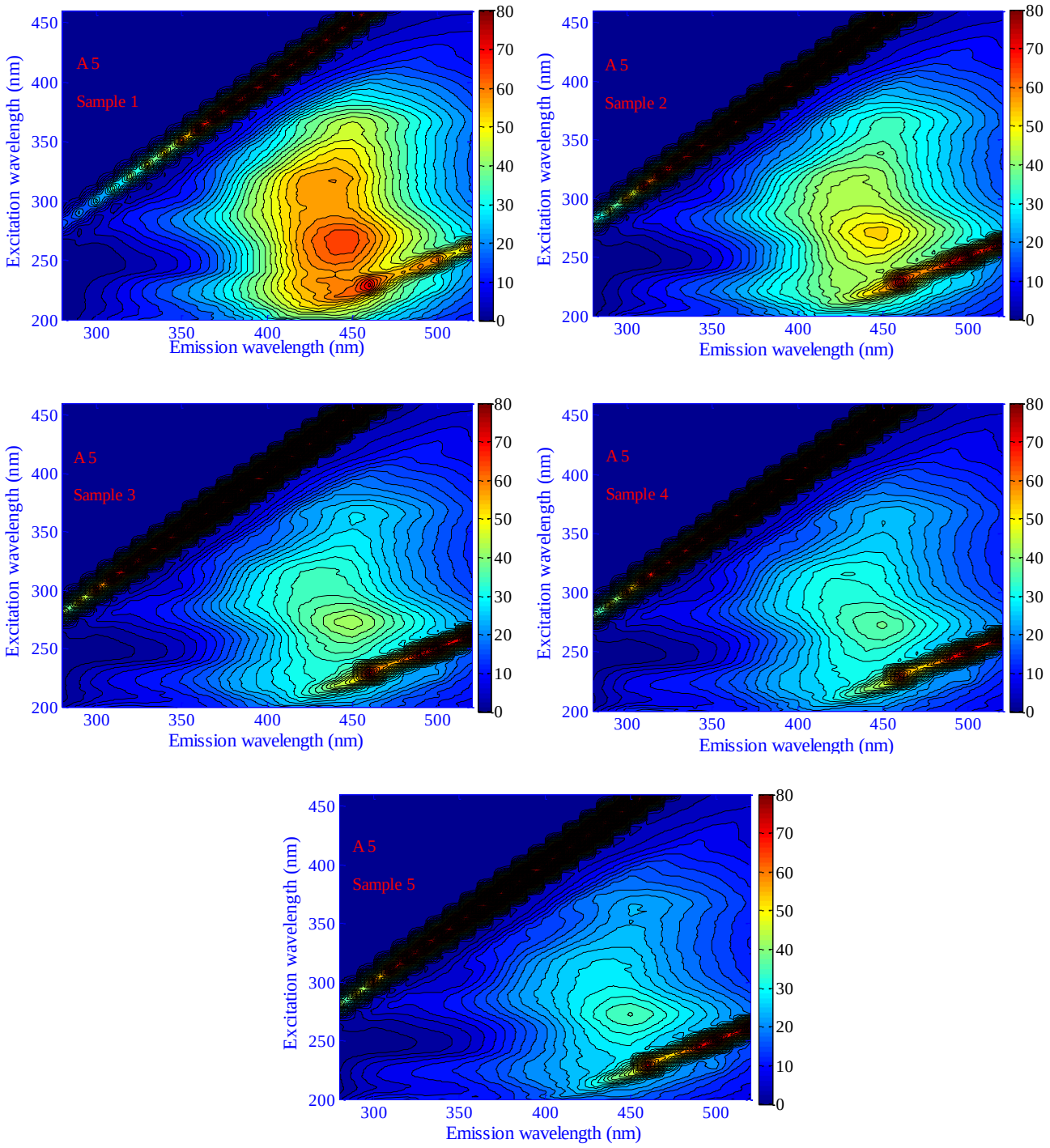

Fig 3-63. EEM contour maps for A 5 during leaching tests.

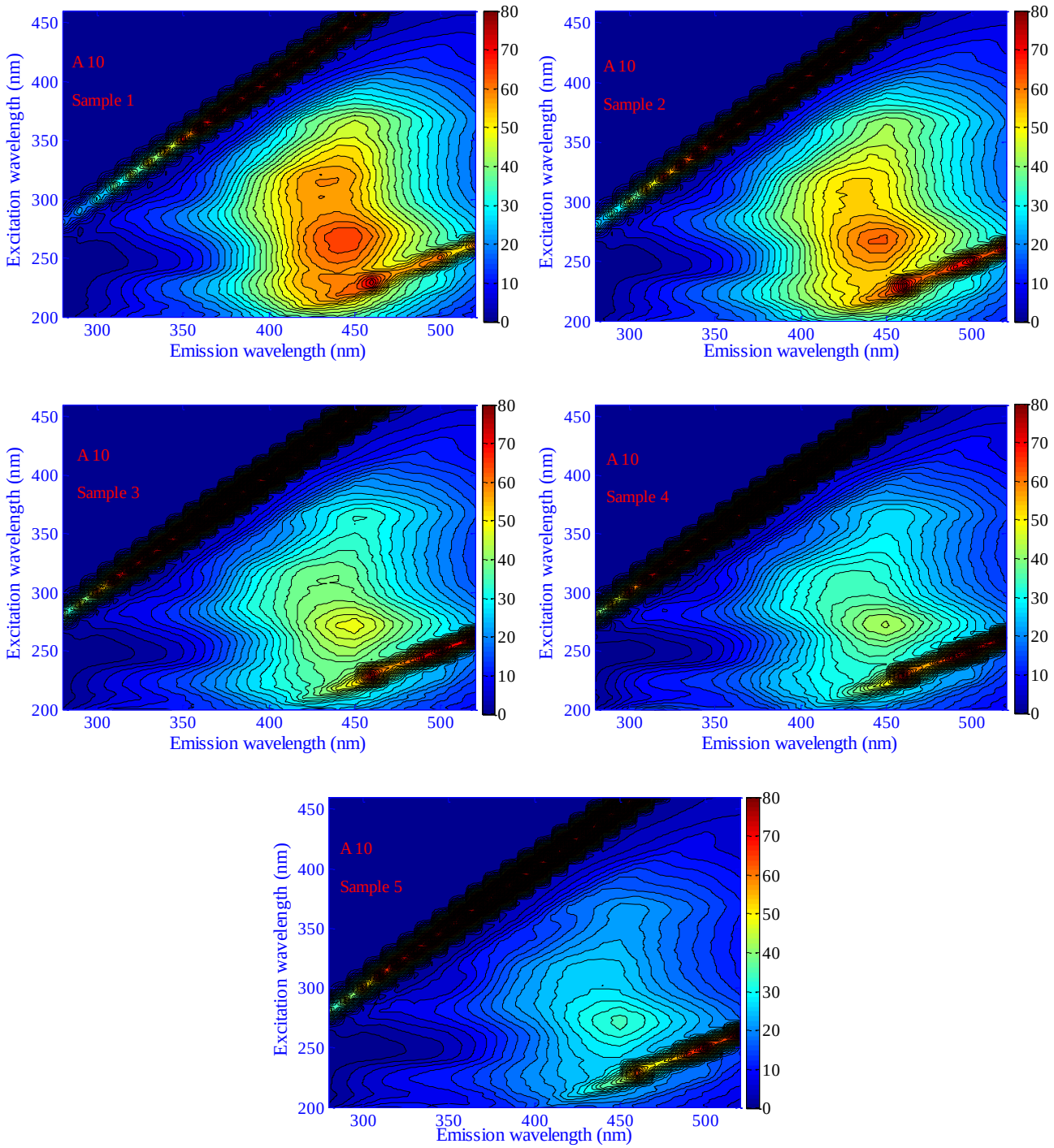

Fig 3-64. EEM contour maps for A 10 during leaching tests.

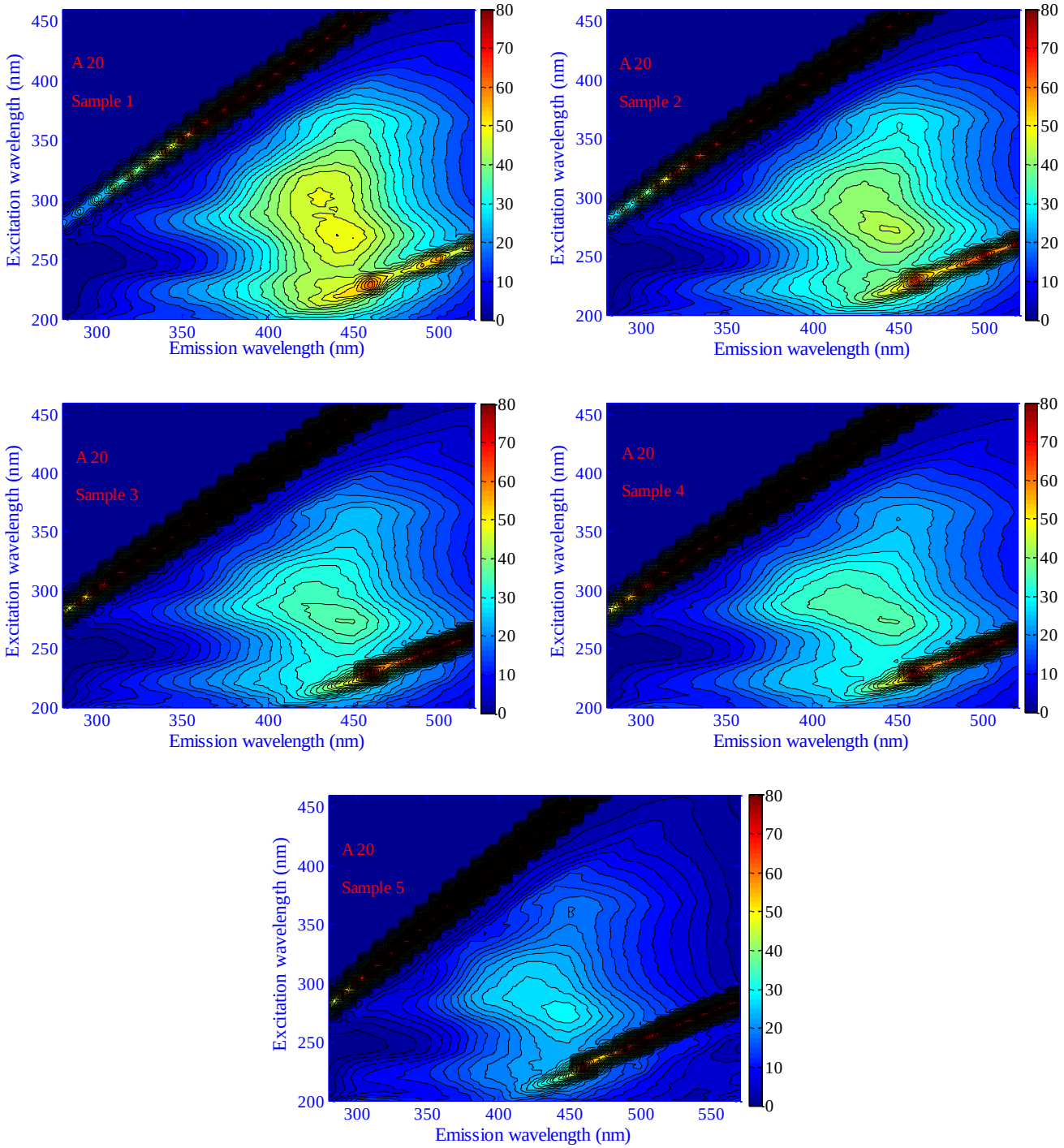

Fig 3-65. EEM contour maps for A 20 during leaching tests.

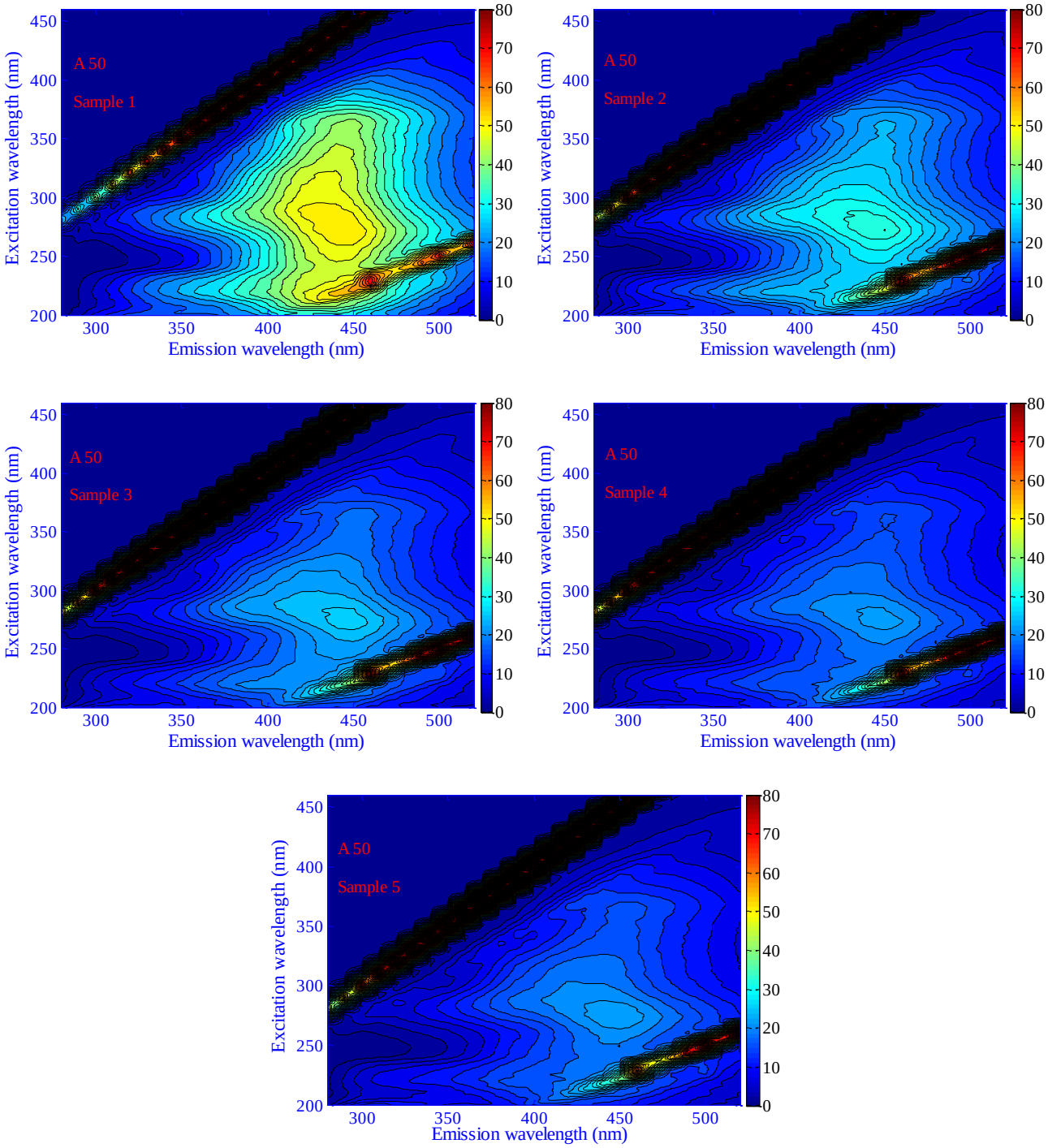

Fig 3-66. EEM contour maps for A 50 during leaching tests.

The total mass (µg) and the percentage of Ag present in DOM leached from the compost samples during the leaching experiment were calculated. The results are given in Table 3-22.

Table 3-22. The total mass (µg) and the percentage (%) of Ag leached from the compost samples.

Sample	Ag (µg)		% Ag
	Av	St Dev	
A 5	0.193	0.032	2.205
A 10	0.372	0.051	1.919
A 20	0.872	0.237	2.417
A 50	2.589	0.504	3.068

The relation of Ag binding to the carbon content of HA was estimated for experiment A 50. The first two leaching samples of A 50, in triplicates, were separated in HA and FA and quantified using fluorescence spectroscopy. The carbon content of HA was found to be around 46%. The carbon content of FA is lower but is unknown. If we assume that FA contains 44% carbon, then we can estimate the total carbon in HS (both FA and HA). The HA, FA, Ag and C concentrations of experiment A 50 for the first two leaching samples are given in Table 3-23.

Table 3-23. HA, FA, Ag and C concentrations of A 50 first two leaching samples in triplicates.

	FA	HA	Ag	C
	(mg/L)	(mg/L)	(µg/L)	(mg/L)
Sample 1 (a)	105.48	59.12	22.25	73.61
Sample 1 (b)	90.73	52.57	21.27	64.11
Sample 1 (c)	63.05	77.42	31.77	63.36
Sample 2 (a)	36.42	16.65	8.72	23.68
Sample 2 (b)	13.53	18.36	8.99	14.40
Sample 2 (c)	10.31	20.24	6.34	13.84

Correlations between HA and Ag concentration in the samples were sought. Figure 3-67 indicates a linear correlation between the HA concentration and Ag concentration in the samples with an R^2 =0.977.

Correlations between carbon and Ag concentration in the samples were also sought. Figure 3-68 suggest that there might be a correlation (R^2 =0.7992) between the carbon content of HS and the amount of Ag that binds to it.

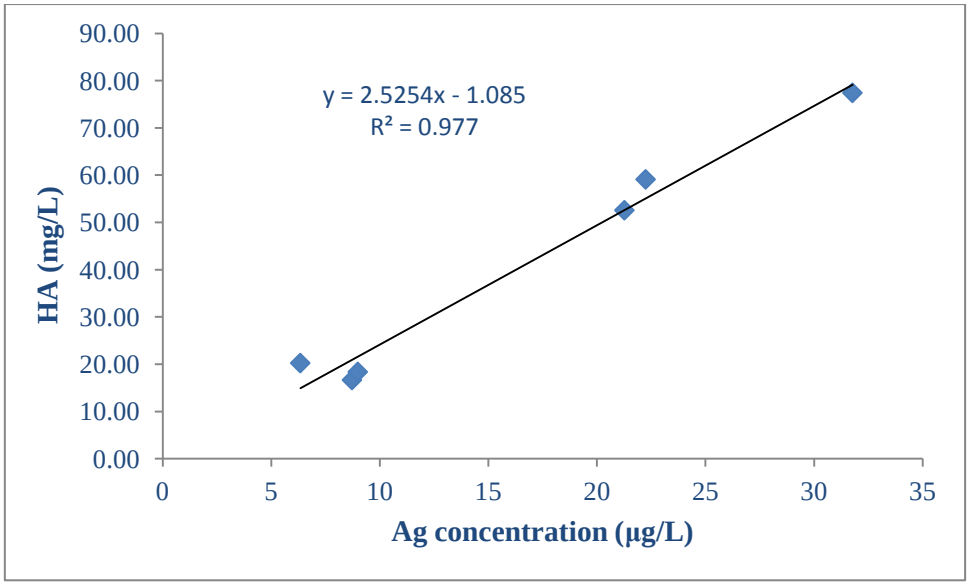

Fig 3-67. HA concentration *vs.* Ag concentration for A 50 first two leaching samples.

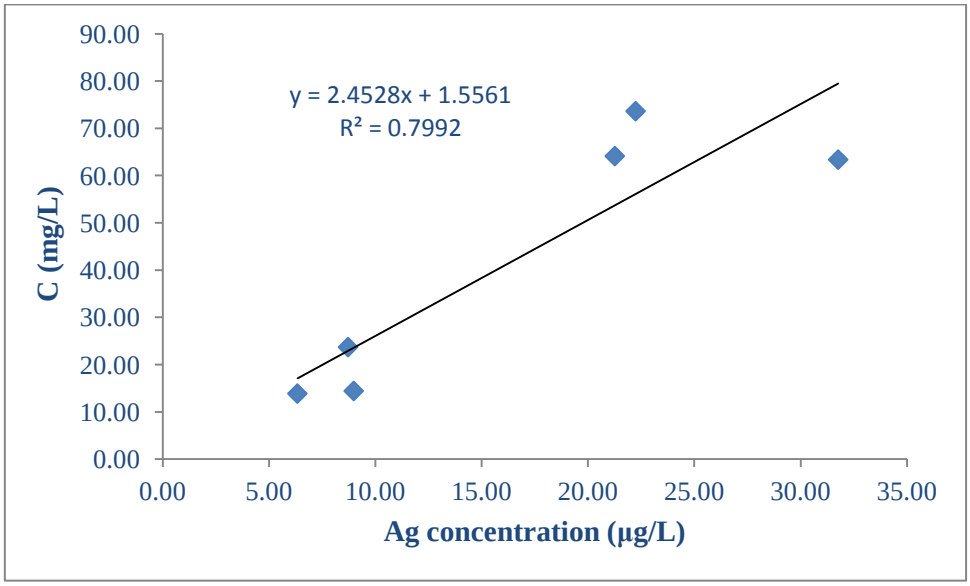

Fig 3-68. Carbon content *vs.* Ag concentration for A 50 first two leaching samples.

The particle size of NPs was estimated for leaching sample 2 of experiment A 50 to elucidate whether aggregation occurred. The sample was analysed using atomic force microscopy. The results are presented in Figure 3-66. The particle size fell in the nano region (1-100nm). Most of the peaks were less than 20nm except of two peaks which appeared around 60 and 95nm respectively. This may indicate that no significant aggregation occurred after leaching of the samples to the environment. These results corroborated previous results presented here and further indicated that HS may act as a stabiliser for the NPs in the solution.

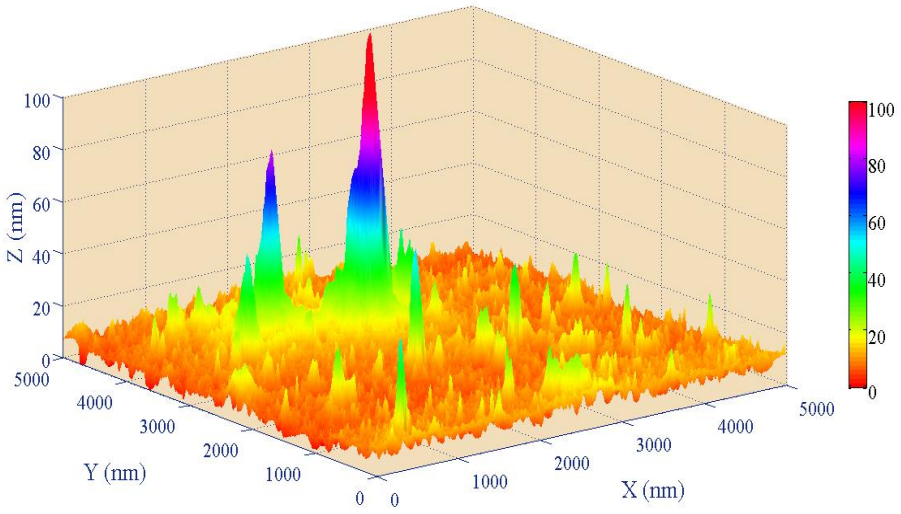

Fig 3-69. Image from atomic force microscopy to detect the particle size of the AgNPs.

3.2.4.2.3 Discussion

FA and HA affect soil fertility and facilitate the transport of contaminants through soils and aquatic media (Ochs, Cosovic et al. 1994). To evaluate the risk of contaminants migration through soil or groundwater it is essential to understand how colloids and associated contaminants behave (Akbour, Douch et al. 2002). The FA and HA contain mainly carboxylic and phenolic acidic groups that are naturally oxidized giving their surface a negative charge in aqueous media. The negative surface area enhances the formation of complexes between acidic groups and divalent cations that may occur in soils. The physicochemical behaviour, mobility and accumulation of FA and HA in soils have been previously related to various parameters such as pH, nature and amount of metal ions present in the medium (Jada, Akbour et al. 2006).

Experiments AT 20 and AT 50 resulted in higher UV absorbance values at λ=254nm in the 1st and 2nd leaching sample (see Figure 3-50) compared to experiments AT 5 and AT 10. This implied that the leaching concentrations of HA and FA were higher for AT 20 and AT 50. This did not happen for experiments A 20 and A 50 where UV absorbance values at λ=254 nm were similar with A 5 and A 10 values. The difference in HA and FA leaching concentrations may be attributed to the formation of complexes between acidic groups and Ag, Ti cations. The formation of such complexes may reduce the negative charge of HA and FA surface and consequently enhance their mobility.

The particle size results indicated that the size fell into the nano-region for both samples analysed. This may have further environmental implications as NPs exhibit higher mobility compared to larger clusters of particles and furthermore their toxicity effects depend on their size. However, the results suggested that only a few percentage of the total NPs amount in compost, up to 5% for Ag and up to 15% for Ti, may be leaching into the environment (see Tables 3-19 and 3-22). These results indicated that NPs mainly will accumulate in soils following application of NP-contaminated compost.

3.2.5 Final remarks

The germination test results suggested that AgNP and Ag-TiO_2NP-contaminated composts have no phytotoxic effects to the cress germination. The leaching test results suggested that only a few percentage of the total NPs amount in compost, up to *ca.* 5% for Ag and up to *ca.* 15% for Ti, is leaching to the environment. This indicated that NPs mainly will accumulate in soils following application of NP-contaminated compost.

CHAPTER 4: LIFE CYCLE ASSESSMENT OF BIODEGRADABLE MUNICIPAL SOLID WASTE COMPOSTING: EFFECT OF SILVER NANOPARTICLES

4.1 Introduction

LCA has been developed as a major tool for sustainability decision support. The LCA study can be conducted on a single system but most of the studies are comparative providing science-based defence to public concerns of a product's environmental performance compared to alternatives. There is no conclusion about the sustainability or the environmental performance of a product (or service) but only an indication if a product is more sustainable or more environmental friendly compared to another product.

The core goal of environmental LCA, as was established in nineties, was to help improve environmental quality with the assumption that improves micro environmental performance of a product-function system corresponds to an environmental improvement at the macro level (Curran 2012).

The International Organization for Standardization (ISO) has issued a series of standards and technical reports for LCA, referred to as the 14040 series. The ISO 14040:2006 (2nd edition) and ISO 14044:2006 replaced ISO 14040:1997, ISO 14041:1998, ISO 14042:2000 and ISO 14043:2000. The principles and framework for LCA is described in ISO 1440:2006 covering both LCA and LCI studies. The ISO 14044:2006 specifies the requirements and provides the guidelines for LCA (ISO 2006a, ISO 2006b).

The LCA framework according to ISO 14044 is presented in Figure 4-1. Four phases are defined: the goal and scope definition, the inventory analysis, the impact assessment and the interpretation phase. There are studies, usually referred as LCI studies, where the goal is satisfied by performing only the inventory analysis and the interpretation phase (ISO 2006b).

In the context of LCA, it is common to distinguish between consequential and attributional modeling. The term consequential LCA (effect-oriented or change-oriented LCA) describes a modelling approach that seeks to describe the consequences of changes or a decision (Weidema B. P. 2009). The attributional

approach aims to account the environmental impacts of a product be investigated (Ekvall T. 2001).

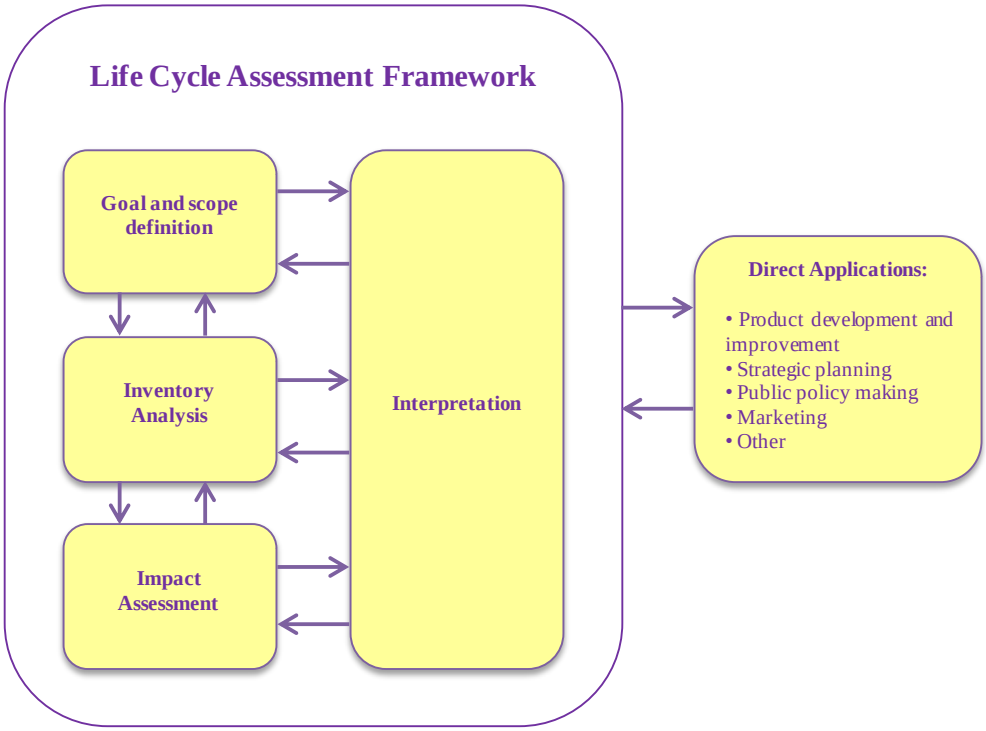

Fig 4-1. LCA framework according to ISO 14044 (ISO 2006b).

4.2 Methodology

4.2.1 Goal and Scope definition

The plan of the LCA study is defined in the first phase by formulating the question and stating the context of answering the question (Curran 2012). The major topics of concern while defining the goal of the study include: the intended application of the results; the reason for carrying out the study and the decision context; the limitations due to the method; assumptions and impact cover; the target audience of the results; the comparative studies to be disclosed to the public and the commissioner of the study. The intended applications can be, for example, comparison of goods and services, weak point analysis of a specific product, identification of Key Environmental Performance Indicators of a product group for Ecodesign, benchmarking of specific products against the product group's average etc. Separate or combined applications might be intended by a study. Different applications often require different methodological approaches for the LCI modeling and sometimes different data background. The identification of the decision context is a key criterion

to choose the LCI modelling framework (attributional or consequential) and the LCI methods approaches (substitution or allocation). Any kind of limitations of the usability of the LCA results shall be clearly identified in the goal definition. Limitations may arise from the applied methodology, limited impact coverage or assumptions made in the study. The initial limitations may need to be adjusted during the later LCA phases when all the related details are clear. It is essential to identify the audience to which the results of the study are intended to be communicated to. The requirements of the study (documentation, review, confidentiality) can be different for different target audiences (i.e. technical vs. non-technical) (JRC 2010a).

The scope definition phase is the detailed definition of the object of the LCI/LCA study. Several scope items shall be defined in detail during this phase. These include the types of the deliverables of the study, the system or process to be studied, the functional unit, the system boundaries, methodological issues (such as allocation procedures), data quality requirements, impact categories to be included, impact assessment methods and need for critical review (Curran 2012). The deliverables can be the LCI study or dataset, the LCIA results, a comparative LCA study, a non-comparative LCA study and a detailed LCI model of the analysed system. The types of deliverables depend on the intended application. For example, the deliverable for the application "comparison of goods and services" is a "comparative LCA study" while the deliverables for the application "weak point analysis of a specific product" are a "non- comparative LCA study" and a "detailed LCI model of system" (JRC 2010a). The system or process to be analysed identified and specified in detail during the scope phase using the system's function, the functional unit and the system's reference flow. The system's function and the functional unit are necessary to ensure the comparability of LCA results. The functional unit names and quantifies the quantitative and qualitative aspects of the function. For example, the comparison of the same material through two different treatments can be "1Kg of material via the first treatment" vs. "1Kg of material via the second treatment" when the functional unit has been quantified to be 1Kg. Qualitative aspects of the functional unit are often necessary to ensure that the compared products are indeed comparable. An example of a qualitative aspect is the users perception of the product as being fashionable or possessing specific design features. The reference flow is the flow to which all other input and output flows quantitatively relate and can be expressed in direct relation to the functional unit or in relation with the product type (i.e. 1Kg product type X). The

choice of the LCI framework and the method approaches to be used depends on the goal of the study and shall be decided early during the scope phase. Attributional and consequential are the two LCI modelling types used in LCA practice. The choice of the appropriate LCI modelling is not covered by the ISO 14044. The attributional LCI modelling describes or accounts (known as well as descriptive or accounting LCI modelling) the potential environmental impacts that can be attributed to a system over its life cycle. The consequential LCI modelling aims to identify the consequences that a decision in the foreground system has for other processes and systems of the economy and models the analysed system around these consequences. A hypothetic (nor the actual) generic supply-chain is modelled including political interactions and consumer behaviour changes (JRC 2010a).

In case of a multifunctional process (several goods or services delivered by the process) the inputs and outputs shall be allocated to the different products. The sum of the allocated inputs and outputs of a unit process shall be equal to the inputs and outputs of the unit process before allocation (ISO 2006b).

Three steps are suggested in ISO 14044 to deal with allocation. The first step to avoid allocation is to divide (subdivision) the unit process into two or more sub-processes and collect the input and output data related to these sub-processes or to expand (system expanding) the product system to include the additional functions related to the co-products. The second step copes with the case that allocation cannot be avoided and suggest the inputs and outputs of the system to be partitioned between the different products or functions in a way that reflects the underlying physical relationships between them. The third step suggests that, where physical relationship alone cannot be established or used as a basis for allocation, the allocation shall be establish in a way that reflects other relationships between inputs/outputs and products or functions (ISO 2006b).

Subdivision, when is applicable, avoids the need for allocation because it divides the multifunctional black box unit processes to mono-functional single operation unit processes. Data are collected individually for each mono-functional process. It is not always possible to use subdivision to avoid allocation. It is the only correct solution under attributional modelling to solve multifunctionality. It can be applied as well to consequential modelling.

System expansion is the addition of a new function by expanding the system boundaries to make the system comparable and avoid allocation. Substitution is the

subtraction of the inventory of another system from the analysed system. System expansion and substitution are used under consequential modelling to solve multifunctionality. Substitution can be applied under attributional modelling.

4.2.2 The functional unit and system boundaries

The functional unit is necessary to ensure the comparability of LCA results as its primary purpose is to provide a reference to which the inputs and outputs are related. The system boundaries must be defined in terms of the life cycle stages to be included in the analysis, the geographic and time boundaries of the analysis and the flows and impact categories to be included (Curran 2012). The system boundaries separate the system from the rest of the technosphere. A decision shall be made of the processes, activities and elementary flows to be included to the analysed system. The definition of the system boundaries has two aspects: a qualitative and a quantitative. All the elementary, waste and non-reference flows that are not quantitatively relevant can be ignored (cut off). The extent of the cut off is acceptable and has no consequences on the validity of the LCA if it is in line with the goal and scope of the LCA study.

The geographic boundaries of the system influence factors such as raw material sourcing, technology used, electricity grids and transportation distances. Geographic boundaries also influence end-of-life scenarios. For example, different countries have different recycling rates, legislation, consumer behaviour, access to waste to energy facilities, etc. End-of-life management practices change over time so time boundaries can have an influence on the relevance of the study results.

4.2.3 Life Cycle Inventory (LCI)

ISO define Life Cycle Inventory Analysis (LCI) as the phase of LCA involving the compilation and quantification of inputs and outputs of a product throughout its life cycle (ISO 2006a). Different unit processes are connected into a system in a LCI created a flow diagram. A unit process in LCI is the smallest element for which input and output data are quantified. For each of the unit process included, quantitative data should be collected. Large amounts of process and production data are needed to complete the LCI. Although, there is a large number of available public databases, at some point it is needed to collect data from original sources. Some examples of data sources include meter readings, laboratory test results, reference books, industry data reports, technical journals, national statistics and government reports (JRC 2010a).

The inventory of flows to and from the natural environment serves as the basis for the impact assessment and the interpretation phases, so it is critically important that the inventory be methodologically sound, complete and unbiased. Although ISO provide detailed guidance on the key aspects of the LCI methodology, in some areas is flexible allowing the practitioner to make methodological choices relevant to the characteristics of the specific system being analysed (JRC 2010a).

4.2.4 Life Cycle Impact Assessment (LCIA)

Life Cycle Impact Assessment (LCIA) phase aims at understanding and evaluating the magnitude and significance of the potential environmental impacts for a product system throughout the life cycle of the product (ISO 2006a). Impact category is the central element in this phase. ISO 14044 defines it as a class representing environmental issues of concern to which LCIA results may be assigned.

According to ISO 14044 (2006), Life Cycle Impact Assessment proceeds through two mandatory and two optional steps. The mandatory steps include: (a) the selection of impact categories and classification and (b) the characterization. The first step includes the definition of the impact categories by their impact pathway and impact indicator and the assignment of the LCI elementary flows to the impact categories according to the substances ability to contribute to different environmental issues. The characterization regards to the conversion of the LCI results to a list of numbers known as “category indicator results” or “score” or LCIA profile or “characterization table” etc. The results are expressed in a unit common to all contributions within the impact category (e.g. kg CO_2-equivalents for greenhouse gases contributing to the impact category climate change) by applying characterization factors. A characterization factor is a substance-specific factor calculated with a characterization model for expressing the impact from the particular elementary flow in terms of the common unit of the category indicator.

The optional steps include the normalization and the weighting. In normalization step, the different characterized impact scores are related to a common reference, e.g. the impacts caused by one person during one year, in order to facilitate comparisons across impact categories. In weighting step, a ranking and/or weighting is performed of the different environmental impact categories reflecting the relative importance of the impacts considered in the study. Weighting may be needed when trade-off situations occur in LCAs used for comparisons.

ISO 14042 does not provide a default list of impact categories. This might be means that the impact categories must be defined anew for each study. A list of impact categories is proposed in each method that facilitates the work of practitioners. The choice is often made on the basis of a recommended impact assessment guidebook (IMPACT 2002+, TRACI, ReCiPe, ILCD, etc) or its implementation in software.

4.2.5 Life Cycle Assessment Methods

Different methods have developed to convert the emission to the environment into impact category indicators. Some of them focus on the midpoint level (acidification, climate change, ecotoxicity), while other focus on the endpoint level (damage to human health).

4.2.5.1 ReCiPe

ReCiPe is one of the most popular method which compromise harmonized category indicators in both midpoint and endpoint level. The method addresses eighteen impact categories at the midpoint level and three impact categories at endpoint level. Impact categories addresses at midpoint level include climate change (CC), ozone depletion (OD), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), human toxicity (HT), photochemical oxidation formation (POF), particulate matter formation (PMF), terrestrial ecotoxicity (TET), freshwater ecotoxicity (FET), ionizing radiation (IR), agricultural land occupation (ALO), urban land occupation (ULO), natural land transformation (NLT), water depletion (WD), mineral resource depletion (MRD) and fossil fuel depletion (FD). Endpoint level include damage to human health (HH), damage to ecosystem diversity (ED) and damage to resource availability (RA) (Mark Goedkoop 2009).

Three perspectives are used to ReCiPe methodology to deal with uncertainty: the Individualist (I), the Hierarchist (H) and the Egalitarian (E). These perspectives group similar types of assumptions and choices. The individualist perspective is based on the short-term interest, impact types that are undisputed, technological optimism and human adaption. The hierarchist perspective is based on the most common policy principles with regards to time frame and other issues. The egalitarian perspective is taking into account the longest time frame and impact types that are not fully yet established but for which some indication is available (Mark Goedkoop 2009). For example, when using the climate change impact category, the time frame for

individualist perspective is 20 y, for hierarchist perspective is 100 y and for egalitarian perspective is 500 y.

Table 4-1. Overview of the midpoint, endpoint categories and indicator names in ReCiPe (Mark Goedkoop 2009).

Midpoint level		
Impact category	**Unit**	**Indicator name**
Climate change (CC)	Kg (CO_2 to air)	Infra-red radiation forcing
Ozone depletion (OD)	Kg (CFC-11 to air)	Stratospheric ozone concentration
Terrestrial acidification (TA)	Kg (SO_2 to air)	Base saturation
Freshwater eutrophication (FE)	Kg (P to freshwater)	Phosphorus concentration
Marine eutrophication (ME)	Kg (N to freshwater)	Nitrogen concentration
Human toxicity (HT)	Kg (14-DCB to urban air)	Hazard-weighted dose
Photochemical oxidation formation (POF)	Kg (NMVOC to air)	Photochemical ozone concentration
Particulate matter formation (PMF)	Kg(PM_{10} to air)	PM_{10} intake
Terrestrial ecotoxicity (TET)	Kg (14-DCB to industrial soil)	Hazard-weighted concentration
Freshwater ecotoxicity (FET)	Kg (14-DCB to freshwater)	Hazard-weighted concentration
Marine ecotoxicity (MET)	Kg (14-DCB to marine water)	Hazard-weighted concentration
Ionizing radiation (IR)	Kg (U^{235} to air)	Absorbed dose
Agricultural land occupation (ALO)	m^2 x yr (agricultural land)	Occupation
Urban land occupation (ULO)	m^2 x yr (urban land)	Occupation
Natural land transformation (NLT)	m^2 x yr (natural land)	Transformation
Water depletion (WD)	m^3 (water)	Amount of water
Mineral resource depletion (MRD)	Kg (Fe)	Grade decrease
Fossil fuel depletion (FD)	Kg (Oil)	Upper heating value
Endpoint level		
Impact category	**Indicator name**	
Damage to human health	Disability-adjusted loss of life years	
Damage to ecosystem diversity	Loss of species during a year	
Damage to resource availability	Increased cost	

4.2.5.2 CML-IA

CML-IA is a LCA methodology developed by the Centre of Environmental Science (CML) of Leiden University in The Netherlands. In original publication "An operational guide to the ISO standards", published in 2001, the authors propose a set of impact categories and characterization methods and factors for the impact assessment step. CML-IA method is defined for midpoint approach.

A default list of impact categories is proposed, distinguishing between baseline impact categories (categories included in almost all LCA studies), study-specific categories (specific categories depending on the goal and scope of the LCA study and whether appropriate data are available) and other impact categories (categories required more elaboration and research before they can use in a LCA study).

Normalization data are available for the Netherlands, Western Europe and Worldwide. Normalization scores are calculated for each baseline indicator and for the reference situations: the World (1990), Europe (1995) and Netherlands (1997) (Huijbregts, Breedveld et al. 2003).

4.2.5.3 Ecological Scarcity 2006

The ecological scarcity method (also called Ecopoints), initially published in 1990, assesses the environmental impacts of Life Cycle Inventories using the "distance to target" principle. The method converts the various environmental impacts into "eco-points" (EP) per unit of pollutant emission or resource extraction. Eco-points result by multiplying the inventory analysis parameters by the respective eco-factor. Eco factors are mainly determined by the current emissions situation. Eco points can be added up to deliver an aggregate score.

The latest method update (2006) introduces a modified eco factor formula. New eco factors are defined for air pollutants (benzene, dioxin and diesel shoot), for endocrine disruptors in waters and discharges of radioactive isotopes to the seas. Bio reactive landfills are now assessed on the basis of the carbon content instead of landfill volume. Land use characterization is based on the impacts of land uses upon plant biodiversity (Frischknecht Rolf 2009).

The mathematical representation of the eco factor is given to the following formula:

$$Eco\ factor = K \cdot \frac{1 \cdot EP}{F_n} \cdot \left(\frac{F}{F_k}\right)^2 \cdot c$$

where $\boldsymbol{K}$ is the characterization factor of a pollutant or a resource, $\boldsymbol{F_n}$ is the normalization flow (current annual flow with Switzerland as system boundary), $\boldsymbol{F}$ is the current flow in the reference area, $\boldsymbol{F_k}$ is the critical flow in the reference area, $\boldsymbol{c}$ is a constant serves to obtain readily presentable numerical quantities and $\boldsymbol{EP}$ is the eco-point (the unit of environmental impact assessed).

For example, the eco factor for cadmium in soils, according to the method, is 310000 EP/ g cadmium. The eco factor for nitrate presence into groundwater is 120 EP/g NO_3-N or 27.1 EP/g NO_3.

4.2.5.4 EDIP 2003

EDIP 2003 methodology published by the Danish Environmental Protection Agency and replaced the EDIP97 methodology which presented in Wenzel et *al.* 1996 and Hauschild et al. 1996. The main difference of the new methodology is that introduces the spatial differentiation regarding the emission and the receiving environment and attempt to include exposure in the characterization modeling of the main non global impact categories. EDIP 2003 can be used both in a site-dependent and a site-generic form. In the site-dependent form the characterization factors are spatially resolve at the level of the countries while in the site-generic form does not take spatial variation into account. The site-dependent form can be used to identify the main sources of spatially variation for non global impact categories and to reduce the variation according to the goal of the study. This is crucial as regional differences in source and receptor characteristics may strongly influence the impact from an emission (Wenzel 1996).

4.2.5.5 Impact 2002+

Impact 2002+ method was originally developed by the Swiss Federal Institute. The methodology links the life cycle inventory results (elementary flows and other interventions) via 14 midpoint categories (human toxicity, respiratory effects, ionizing radiation, ozone layer depletion, photochemical oxidation, aquatic ecotoxicity, terrestrial ecotoxicity, terrestrial acidification/eutrophication, land occupation, global warming, non-renewable energy and mineral extraction) to 4 damage categories (human health, ecosystem quality, climate change and resources) (Jolliet O. 2003).

The method provides characterization factors for almost 1500 different LCI results. The characterization factors for human toxicity and aquatic and terrestrial toxicity are taken from the methodology IMPACT 2002-IMPact Assessment of Chemical Toxics (Pennington, Margni et al. 2005) while for the other categories are adapted from existing characterizing methods such as Eco-indicator 99, CML 2001, IPCC and the Cumulative Energy Demand.

Endpoint and midpoint factors are all normalized in respect to the overall endpoint results. Only aquatic acidification and eutrophication is expressed in Kg-equivalents of reference substance because the link to the endpoint is not scientifically established. The normalization factors for the 4 damage categories are given in table 4-2.

Table 4-2. Normalization factor of damage categories for Impact 2002+ method (Jolliet, Margni et al. 2003).

Damage categories	Normalization factors	Unit
Human Health	0.0071	DALY/pers/yr
Ecosystem Quality	13700	PDF.m^2.yr/pers/yr
Climate change	9950	Kg CO_2/pers/yr
Resources	152000	MJ/pers/yr

4.2.6 Ecoinvent v.3 database

The ecoinvent database was developed by the Swiss Centre of Life Cycle Inventories known as the Ecoinvent Centre. The first version v1 was released in 2003. The first extension and revision of the contents of the database, ecoinvent v.2, was released in 2007 and the latest, ecoinvent v. 3, was released in 2013.

The impact assessment factors proposed from each method (e.g., CML, ReCiPe) are linked to the ecoinvent data in order to facilitate the usage and avoid discrepancies due to misunderstandings or different interpretations of the original reports.

The LCI datasets in ecoinvent are interlinked so all intermediate goods and services inputs to a unit process (electricity consumption, use of capital equipment etc.) are linked to other unit processes that supply these intermediate goods and processes (Weidema B P 2011). Any change in one unit process dataset influence the accumulated LCI results of almost all other datasets.

The ecoinvent database contains LCIA results to facilitate the interpretation of the LCI results but assumptions and interpretations are necessary to match the current LCIA methods with the ecoinvent inventory results.

The ecoinvent data are available in different implementations representing different system models such as undefined, system models with substitution (consequential) and system models with partitioning (allocation). Undefined system model represent original activity datasets as obtained and entered by the data providers. These datasets are useful for investigating the environmental impacts of a specific activity without regard to its upstream or downstream impacts. Consequential system model is used in LCA studies that investigate the long-term consequences of small scale decisions. This system model uses substitution (or system expansion) to substitute by-products outputs and includes only activities that expected to change in long-term as a consequence of a small-scale change in demand taking into account both constrained markets and technology constraints. The allocation system model subdivides multi-

product activities by allocating all flows relative to their "true value", which is the economic revenue corrected for some market imperfections and fluctuations and includes activities in proportion to their current production volume.

4.2.7 LCA Software

LCA is an approach to compile and evaluate all inputs, outputs and potential environmental impacts related to a product or service, over its entire lifecycle (ISO 2006a). A LCA study requires information about a large number of processes, their input and output flows, and all these processes need to be connected and a balance is calculated. It is not unusual to find 500 unit processes and 1000 environmental flows in a typical LCA. Using a spreadsheet to perform the analysis is usually inefficient for performing the analysis in case of a large number of system units. Using a commercial LCA software having a database of process data and impact assessment data, the practitioner can perform a detailed analysis, including sensitivity and uncertainty analysis. LCA software includes different approaches or models to perform the calculations, assess the environmental impacts and provide the results to the user.

SimaPro is one of the most popular LCA software. The main advantage of this software is the ability to deal with a huge number of unit processes in one calculation. The calculations are achieved following the links of a unit process to another dynamically. A major advantage of the dynamic linking is that the results always reflects the recent database status.

A variety of life cycle assessment methods are available to choose from in SimaPro 8. Midpoint assessment methods include ReCiPe, USEtox, EDIP 2003, IPCC 2007, CML IA, Traci 2, BEES, Ecological Scarcity 2006 etc. Endpoint assessment methods include ReCiPe, Eco-indicator 99, EPS 2000 and Impact 2002+.

The basic structure of impact assessment methods in SimaPro is:

- Characterization. The substances that contribute to an impact category are multiply with a characterization factor that expresses the relative contribution of the substance.
- Damage Assessment. The purpose of the damage assessment is to combine a number of impact category indicators into a damage category.

- Normalization. The impact category is divided by a reference value to be compared. All the impact categories get the same unit which makes it easier to compare. Normalization can be applied in both characterization and damage assessment results.
- Weighting. The impact category indicator results are multiplied by weighting factors and added to create a total or single score.

4.2.8 Uncertainty Analysis

LCA practitioners assign single values to model parameters, build deterministic models to approximate environmental outcomes and report results as point estimates. This approach fails to capture the variability and uncertainty inherent in LCA and reduces the effectiveness of LCA to support decision making (Lloyd and Ries 2007). The term uncertainty generally refers to random errors such as imprecision in measurements while variability accounts for stochastic variation in data such as seasonal and spatial variation of a parameter (Geisler, Hellweg et al. 2005). The quantification of uncertainty and variability through LCA study is an important step towards reliable and transparent decision support (Geisler, Hellweg et al. 2005).

Uncertainty in LCA has been distinguished between: parameter uncertainty due to imprecise knowledge of LCI and LCIA parameters such as mass flows and substance properties; temporal and spatial variability in LCI and LCIA parameters; variability between sources in LCI and objects of the assessment in LCIA; uncertainty in the models used and uncertainty due to the choices in LCA such as which allocation method to apply (Huijbregts 1998).

Monte Carlo simulation is used to deal with uncertainty and variability in parameters whereas the LCA results are calculated for different scenarios to deal with model and choices uncertainty (Geisler, Hellweg et al. 2005).

4.2.9 Life Cycle Sustainability Analysis

Life Cycle Sustainability Analysis (LCSA) has mainly been conceptualised as a combination of LCA, social LCA and environmental life cycle costing (Cinelli, Coles et al. 2013). This concept requires three separate assessments, one of each sphere of sustainability, which applied at product level under specific consistency requirements and independently one from other.

The development of a life-cycle based analysis for sustainability needs to move into two main directions: to deepen the scope of mechanisms and to broaden the scope of indicators and/or the object of analysis (Curran 2012). Deepening can be achieved by including physical, social, economic, cultural and political mechanisms in analysis apart of the technological and environmental ones or sophisticating the modelling by adopting spatially differentiated models for non global categories. Broadening the object of analysis is related to the question while broadening the spectrum of indicators is related to the results and can be achieved by extending them in numbers or by including economic and social ones in the analysis.

Consequential LCA (C-LCA) is a clear example of how deepening can be achieved in LCA. C-LCA is dealing with non-linear relationships, hybrid approaches, temporal differentiation, adding optimization strategies and the numerous developments occurred in impact assessment, ranging from regionalization to the development of new characterization methods and adding expert knowledge (Curran 2012).

4.3 Life Cycle Assessment of Compost produced using NP-contaminated Biodegradable Municipal Solid Waste

4.3.1 Introduction

LCA has been successfully used to compare product or services. The NP-contaminated compost containing different types or concentrations of NPs can be regarded as different products and compared using a LCA study because the environmental impacts that can cause are expected to be different. A consequential LCA can be applied to investigate the impact of the NP-contaminated compost in human health, ecosystems and natural resources damage categories.

4.3.2 Goal and Scope Definition

The main goal of this assessment is to investigate the positive and negative environmental impacts of compost due to the presence of AgNPs and Ag-TiO_2NPs in waste streams.

The functional unit is defined as 1 ton compost on dry basis. The system boundaries of this study have been defined as presented in Figure 4-2. The system boundaries include the bio-waste collection, compost production, transportation to field,

application to agricultural production systems and post-application impacts. MSW collection and separation are beyond the scope of the current study. The geographic boundaries are confined to EU-28 and the relevant time is considered to be the present.

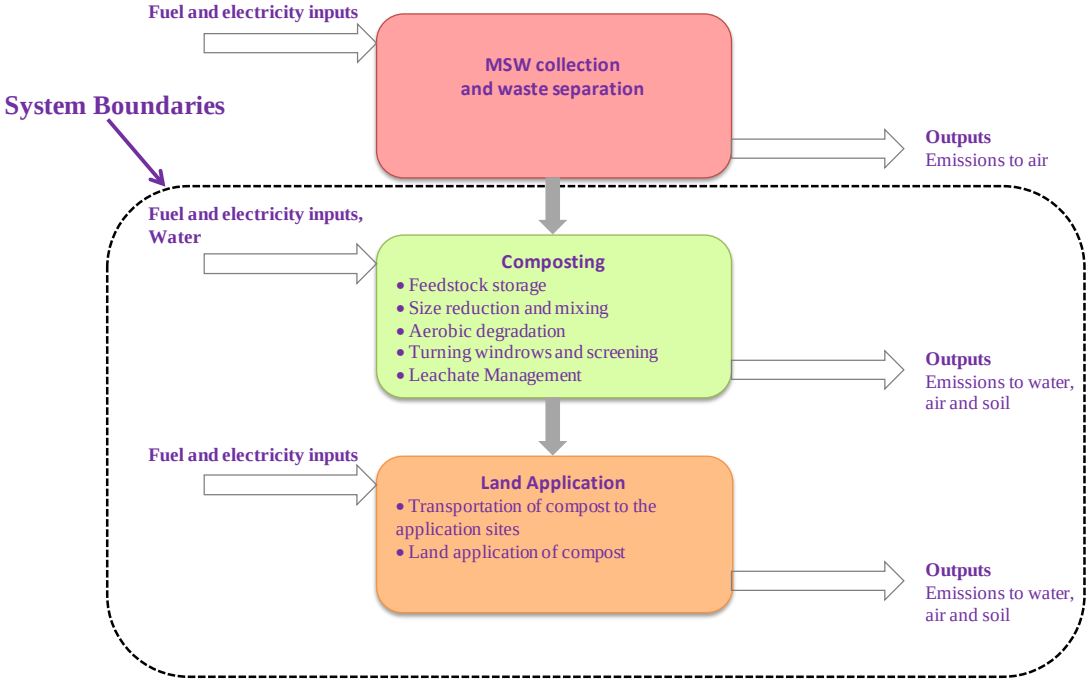

Fig 4-2. System boundaries to analyse environmental aspects of compost production and application to agricultural site.

4.3.3 Life Cycle Inventory

The LCI was compiled using ecoinvent data, literature data and data derived from laboratory results. The literature data and laboratory results concerns the compost benefits and risks. The literature data were used to calculate the carbon sequestration and the avoided amount of fertilizers due to the compost application to soil and the laboratory results were used to calculate the NP-emissions to soil and surface/ground waters. The activities derived from ecoinvent database are the compost production, the machinery used for compost application and the transportation.

4.3.3.1 Bio-waste composting

The bio-waste composting inventory refers to 2.5 ton fresh weight of compost (1 ton dry weight) produced in an open composting plant at Switzerland. The activities accounted include: the infrastructure of the composting plant; the energy demand for

operating the plant; the emissions and the transportation related to the collection of bio-waste.

4.3.3.2 Transportation of compost to agricultural site

LCI data on transportation of compost to the application site are based on vehicle type, road type and distance travelled in ecoinvent database. Three types of vehicles for road transport are used: small trucks (<10 t) with an average load capacity of 3 tones; medium sized trucks (10-20 t) with an average load capacity of 6.2 tones and large trucks (>20 t) with an average load capacity of 24 tones. Load Factor (LF) is defined as the percentage of the load capacity that is been occupied. Three types of roads are defined: urban area, country roads and highways. The emissions calculation is based on data of 2010 where trucks spent 17.5% of their distance in urban areas, 22.1% of their distance on country roads and 60.4% of their distance on highways.

The European emission standards EURO 1-5 which define the acceptable limits for exhaust emissions are used in ecoinvent database. The emissions decrease from EURO 1 to EURO 5 and include emissions from nitrogen oxides (NO_X), carbon monoxide (CO) and particulate matter.

In ecoinvent database, there are two options related to the return trip. The one option is when a vehicle make a complete return trip. This means that the emissions include complete empty return trip of the same distance but instead of the load factor which applied to the first trip, the load factor for the return trip is 0%. The other option is when the vehicle is not directly reloaded on the site of the first destination. The vehicle can drive a couple of kilometres to another location to pick up a new load or might have to drive a long distance before loading a new load. In this case, it is assumed that 20% of the emissions of the first trip is dedicated to the return trip.

In the context of this study, we assume that we use a EURO 5 large truck (24 t average load capacity) with 80% load factor which return empty from the agricultural site.

4.3.3.3 Compost application to soil

Compost is added to soil using mobile machinery such as a manure spreader or a tractor. In the context of this study, it is assumed that the compost is applied to soil using a solid manure spreader loading with a hydraulic loader. The working load of

the machine is 5 t. The functional unit is 1 kg of compost loaded and spreaded and the operation time is 0.00019 h/kg

The inventory includes the diesel fuel consumption and the amount of agricultural machine used. The activity includes the preliminary work at the farm, the transfer from farm to the field (with an assumed distance of 1 km), the field work and the transfer of the machine back to farm.

4.3.3.4 Benefits and risks of compost application to soil

The application of mature compost to soils may result in reduce water use, reduced fertilizer, herbicide, pesticide, lime and gypsum requirements, improved soil properties, carbon sequestration, reduced soil management and maintenance, reduced nutrient loss, reduced eutrophication of waterways etc. Potential risks related to the use of poor quality and/or immature compost include phytotoxicity, physical and chemical contamination, odours, transfer of pathogens to plants and animals, nitrogen drawdown etc.

Most of the benefits due to the compost application to soil are difficult to quantify. For example, the resulted reduced water use is very important in agriculture where water is usually a limiting factor. The quantification of potential benefits due to the compost application on water use efficiency is complex including parameters such as soil type, climate, product type, application rate and crop parameters.

In the context of this study only the benefits from avoided fertilizers and carbon sequestration are accounted as benefits and only the NPs emissions to soil and water are accounted as risks.

4.3.3.4.1 Carbon sequestration in soils

The amount of organic carbon stored in soils results from the net balance between the rate of SOC inputs and rate of mineralization (Post and Kwon 2000). The increase of SOC following application of compost is sequestered carbon, not contributing to greenhouse gases in the atmosphere (DEC 2006). Organic carbon content of humic substances represents the most persistent pool of SOC with mean residence times of several hundreds of years (Piccolo 1996). The carbon storage rates decline with time after initial application and varies from about 0.08 metric tons carbon equivalent per wet short ton organics immediately after compost application to about 0.02 metric ton carbon equivalent per wet short ton organics 24 years after the last application

(USEPA 2002). Carbon binding to soil following compost application has been reported to be 9-14% depending on the soil type and crop rotation (Bruun, Hansen et al. 2006).

In the context of this study we assume that the carbon sequestration is 11% of the total carbon present in compost. The percentage of carbon in compost has been determined around 45% and the ash content around 8.5%. With these assumptions, the amount of carbon sequestered in soils following application of compost will be 40.15 kg per ton of dry compost or 160.6 kg CO_2.

4.3.3.4.2 Avoided production of fertilizer

The production and use of inorganic fertilizers may result in significant environmental impacts such as resource depletion, eutrophication, human toxicity, ecotoxicity and greenhouse gas emissions. The use of compost as fertilizer in agriculture may reduce environmental impacts such as those described above. The replacement of inorganic fertilizer by composted products may not be practical due to the low nutrient content and slow mineralization potentials of macro nutrients in compost (Sikora and Enkiri 2001). Furthermore, high compost application rate can cause risks such as heavy metals accumulation to soils and nitrate leaching to ground waters.

The organic nitrogen mineralization rate in soils is low. It has been reported that N recovery in soil after 33 weeks when applying MSW compost was 22% of the total N and 15% of the organic N while the recovery rate was independent to soil and compost application rate and only depended to C:N ratio (Hadas and Portnoy 1997).

In ecoinvent database, it is assumed that the application of compost for fertilizer use provides 0.7% N, 0.4% P_2O_5 and 0.6% K_2O. The fertilizers types and quantities avoided due to the application of 1 ton of compost to agricultural site are given in Table 4-3.

Table 4-3. Fertilizer types and quantities avoided due to the application of 1 ton of compost, on a dry basis, to agricultural site.

Fertilizer type	Quantity
Phosphate fertilizer as P_2O_5	4 kg
Potassium fertilizer as K_2O	6 kg
Nitrogen fertilizer as N	7 kg

4.3.3.4.3 Emissions to soil, surface and ground waters.

The data of NP emissions to soil and surface/ground waters obtained from the leaching results (see sub-chapter 3.2.4.2) for the various concentrations and types of NPs. The results are summarized in Table 4-4 for emissions to soil and in Table 4-5 for emissions to ground/surface waters.

Table 4-4. Emissions of AgNPs and Ag-TiO_2NPs to soil following application of NP-contaminated compost to agricultural site.

	Ag (mg/kg)		Ti (mg/kg)	
	Average	**StDev**	**Average**	**StDev**
A 0	n.d.	n.d.	n.d.	n.d.
A 5	8.548	0.032	n.d.	n.d.
A 10	19.018	0.051	n.d.	n.d.
AT 0	n.d.	n.d.	n.d.	n.d.
AT 5	7.474	0.003	340.44	0.083
AT 10	16.074	0.024	725.195	0.867
n.d.: not detected				

Table 4-5. Emissions of AgNPs and Ag-TiO_2NPs to surface and ground waters following application of NP-contaminated compost to agricultural site.

	Ag (mg/kg)		Ti (mg/kg)	
	Average	**StDev**	**Average**	**StDev**
A 0	n.d.	n.d.	n.d.	n.d.
A 5	0.193	0.032	n.d.	n.d.
A 10	0.372	0.051	n.d.	n.d.
AT 0	n.d.	n.d.	n.d.	n.d.
AT 5	0.206	0.003	5.075	0.082
AT 10	0.236	0.024	8.775	0.867
n.d.: not detected				

4.3.4 Life Cycle Impact assessment

The comparison of NP-contaminated compost was carried out using a consequential LCA to investigate the impacts of NP-contaminated compost. The consequential LCA provides information about the consequences of changes in the level of output of a product including effects both outside and inside the life cycle of the product (Brander M 2009). The method used for the calculations was the ReCiPe for Europe using the Hierarchist (H) and Egalitarian (E) perspectives. The H perspective is based on the most common policy principles with regards to the time frame. The E perspective is taking into account the longest time frame and impact types that are not fully yet established but for which some indication is available (Mark Goedkoop 2009).

4.3.4.1 Comparison of AgNPs contaminated compost

Three concentrations of AgNPs (A 0, A 5, A 10) in compost were used for the calculations. The LCA study was performed using SimaPro 8 software with ecoinvent v3 database. The results calculated using ReCiPe methodology for Europe using H and E perspectives.

4.3.4.1.1 Results calculated using Hierarchist perspective

The single score of the midpoint impact categories according to the ReCiPe methodology, using consequential modelling with H perspective, for the production of 1 ton compost from bio-waste and the application to agricultural site is given in Table 4-6 while the severity of the impact categories is presented in Figure 4-3.

The overall score was calculated to be 7.74 Pt for non-contaminated compost (A 0) and increased to 12.81 Pt for A 5 and 19.01 Pt for A 10. The presence of AgNPs in compost resulted in a significant effect in terrestrial eco-toxicity ranging as the score increased from -0.00777 Pt for A 0 to 2.62 Pt and 5.84 Pt for A 5 and A 10, respectively. The lower score was calculated for human toxicity and it can be attributed to the avoided production of fertilizers and carbon sequestration. The beneficial effect to human toxicity is eliminated due to the presence of AgNPs as the score is increased from -1.60 Pt for A 0 to 0.83 Pt and 3.82 Pt for A 5 and A 10 respectively. Small differences resulted in scores for marine eco-toxicity and freshwater eco-toxicity due to the presence of NPs in compost.

Table 4-6. Score of midpoint impact categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost and application to agricultural site.

Impact category	Unit	A 0	A 5	A 10
Total	Pt	7.74	12.81	19.01
Climate change Human Health	Pt	5.92	5.92	5.92
Ozone depletion	Pt	-0.00007	-0.00007	-0.00007
Human toxicity	Pt	-1.60	0.83	3.82
Photochemical oxidant formation	Pt	0.00063	0.00063	0.00063
Particulate matter formation	Pt	2.15	2.15	2.15
Ionising radiation	Pt	0.00371	0.00371	0.00371
Climate change Ecosystems	Pt	3.72	3.72	3.72
Terrestrial acidification	Pt	0.03603	0.03603	0.03603
Freshwater eutrophication	Pt	-0.00487	-0.00487	-0.00487
Terrestrial eco-toxicity	Pt	-0.00777	2.62	5.84
Freshwater eco-toxicity	Pt	-0.00484	-0.00423	-0.00353
Marine eco-toxicity	Pt	-0.00097	-0.00090	-0.00082
Agricultural land occupation	Pt	0.14629	0.14629	0.14629
Urban land occupation	Pt	0.04271	0.04271	0.04271
Natural land transformation	Pt	-0.03188	-0.03188	-0.03188
Metal depletion	Pt	-1.37	-1.37	-1.37
Fossil depletion	Pt	-1.24	-1.24	-1.24

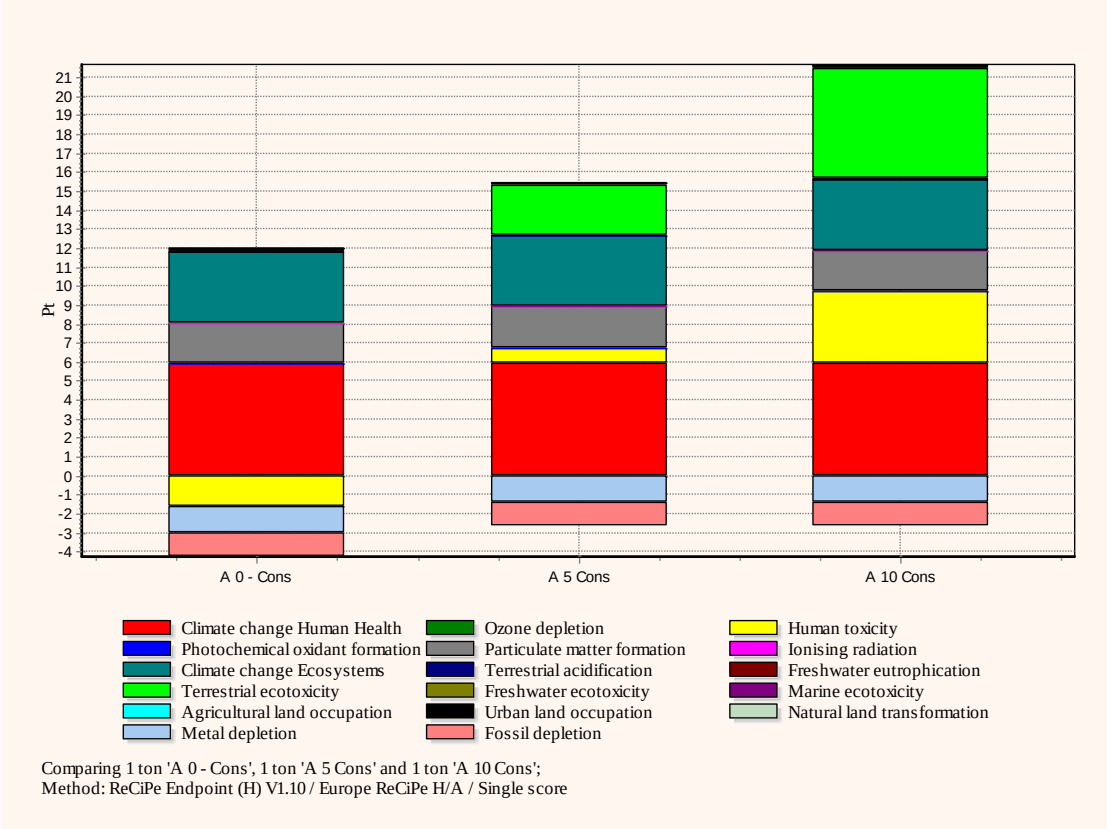

Fig 4-3. Severity of impact categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost and application to agricultural site.

The score of the aggregated damage categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost from bio-waste and the application to agricultural site is given in Table 4-7 while the severity of the aggregated damage categories is presented in Figure 4-4.

The highest score was calculated for human health category for A 10 (11.88 Pt) compost followed by A 5 (8.90 Pt) and A 0 (6.46 Pt). The score of the ecosystems category increased from 3.90 Pt for A 0 to 6.53 Pt and 9.74 Pt for A 5 and A 10 respectively. The score of resources category was the same for the three types of compost.

Table 4-7. Score of aggregated damage categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost and application to agricultural site.

Damage category	Unit	A 0	A 5	A 10
Total	Pt	7.74	12.81	19.01
Human Health	Pt	6.46	8.90	11.88
Ecosystems	Pt	3.90	6.53	9.74
Resources	Pt	-2.61	-2.61	-2.61

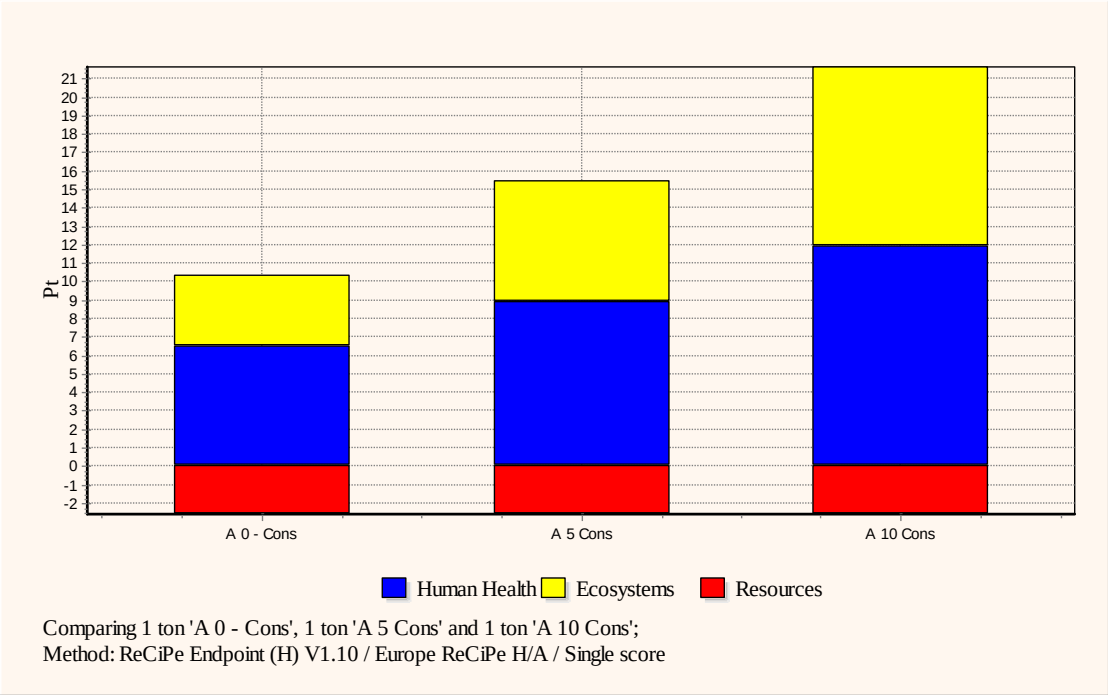

Fig 4-4. Severity of aggregated damage categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost and application to agricultural site.

4.3.4.1.2 Results calculated using Egalitarian perspective

The single score of the midpoint impact categories according to the ReCiPe methodology, using E perspective, for the production of 1 ton compost from bio-waste and the application to agricultural site is given in Table 4-8 while the severity of the impact categories is presented in Fig 4-5. The overall score was calculated to be -30.85 Pt for non-contaminated compost (A 0) and increased to -21.94 Pt for A 5 and -11.04 Pt for A 10. The presence of AgNPs in compost resulted in a significant effect in terrestrial ecotoxicity as the score increased from -0.05878 Pt for A 0 to 5.17 Pt and 11.57 Pt for A 5 and A 10 respectively. The lowest score was calculated for human toxicity category. The beneficial effect to human toxicity is reduced due to the presence of AgNPs as the score increased from -30.69 Pt for A 0 to -27.05 Pt and -22.59 Pt for A 5 and A 10 respectively. Small differences resulted in scores for marine eco-toxicity and freshwater eco-toxicity due to the presence of NPs in compost.

Table 4-8. Score of impact categories according to the ReCiPe methodology for the production of 1 ton compost from bio-waste and application to agricultural site.

Impact category	Unit	A 0	A 5	A 10
Total	Pt	-30.85	-21.94	-11.04
Climate change Human Health	Pt	0.11454	0.11454	0.11454
Ozone depletion	Pt	-0.00004	-0.00004	-0.00004
Human toxicity	Pt	-30.69	-27.05	-22.59
Photochemical oxidant formation	Pt	0.00031	0.00031	0.00031
Particulate matter formation	Pt	1.05	1.05	1.05
Ionising radiation	Pt	0.00182	0.00182	0.00182
Climate change Ecosystems	Pt	0.09626	0.09626	0.09626
Terrestrial acidification	Pt	0.07059	0.07059	0.07059
Freshwater eutrophication	Pt	-0.00320	-0.00320	-0.00320
Terrestrial ecotoxicity	Pt	-0.05878	5.17	11.57
Freshwater ecotoxicity	Pt	-0.00325	-0.00217	-0.00086
Marine ecotoxicity	Pt	-0.66646	-0.62617	-0.57793
Agricultural land occupation	Pt	0.09629	0.09629	0.09629
Urban land occupation	Pt	0.02811	0.02811	0.02811
Natural land transformation	Pt	1.72	1.72	1.72
Metal depletion	Pt	-1.37	-1.37	-1.37
Fossil depletion	Pt	-1.24	-1.24	-1.24

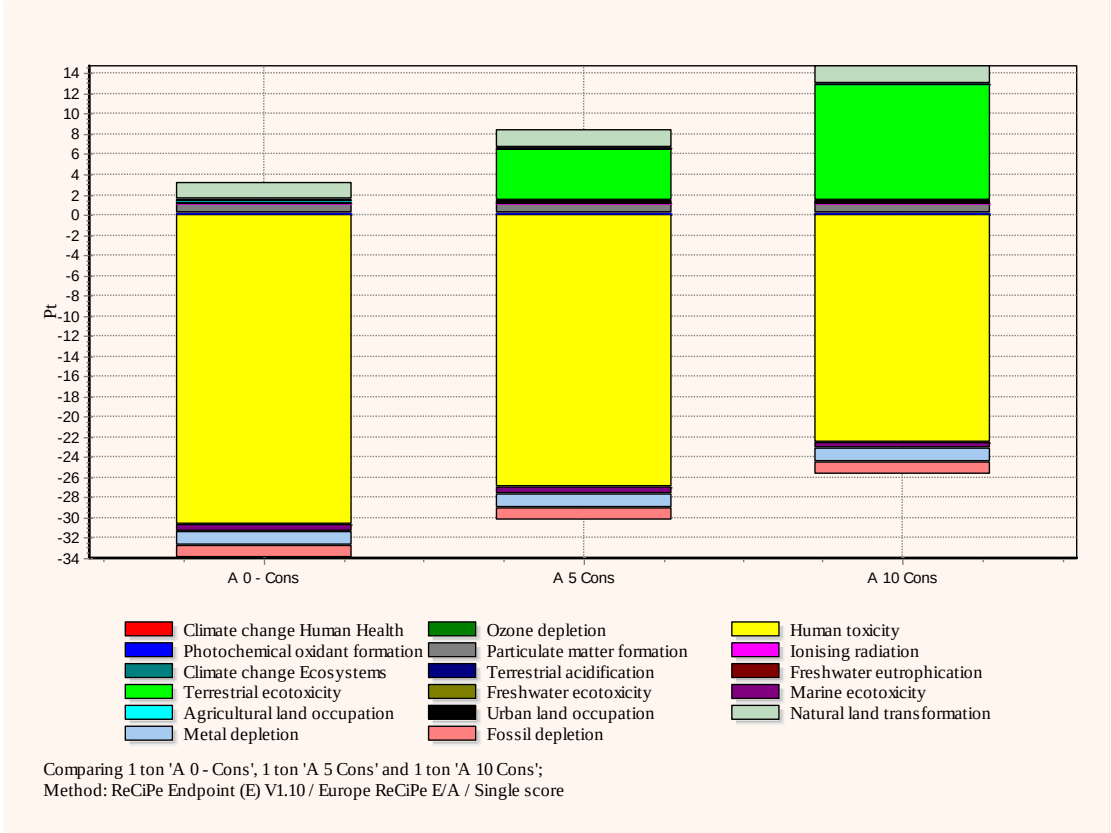

Fig 4-5. Severity of impact categories according to the ReCiPe methodology for the production of 1 tn compost from biowaste and application to agricultural site.

The score of the aggregated damage categories according to the ReCiPe methodology, using E perspective, for the production of 1 ton compost from bio-waste and the application to agricultural site is given in Table 4-9 while the severity of the aggregated damage categories is presented in Fig 4-6.

The highest score was calculated for ecosystems category for A 10 (13.00 Pt) compost followed by A 5 (6.55 Pt) and A 0 (1.28 Pt). The beneficial effect to human health category is reduced due to the presence of AgNPs as the score is increased from -29.52 Pt for A 0 to -25.88 Pt and -21.42 Pt for A 5 and A 10 respectively. The score of resources category was the same for the three types of compost.

Table 4-9. Score of aggregated damage categories according to the ReCiPe methodology for the production of 1 ton compost from bio-waste and application to agricultural site.

Damage category	Unit	A 0	A 5	A 10
Total	Pt	-30.85	-21.94	-11.04
Human Health	Pt	-29.52	-25.88	-21.42
Ecosystems	Pt	1.28	6.55	13.00
Resources	Pt	-2.61	-2.61	-2.61

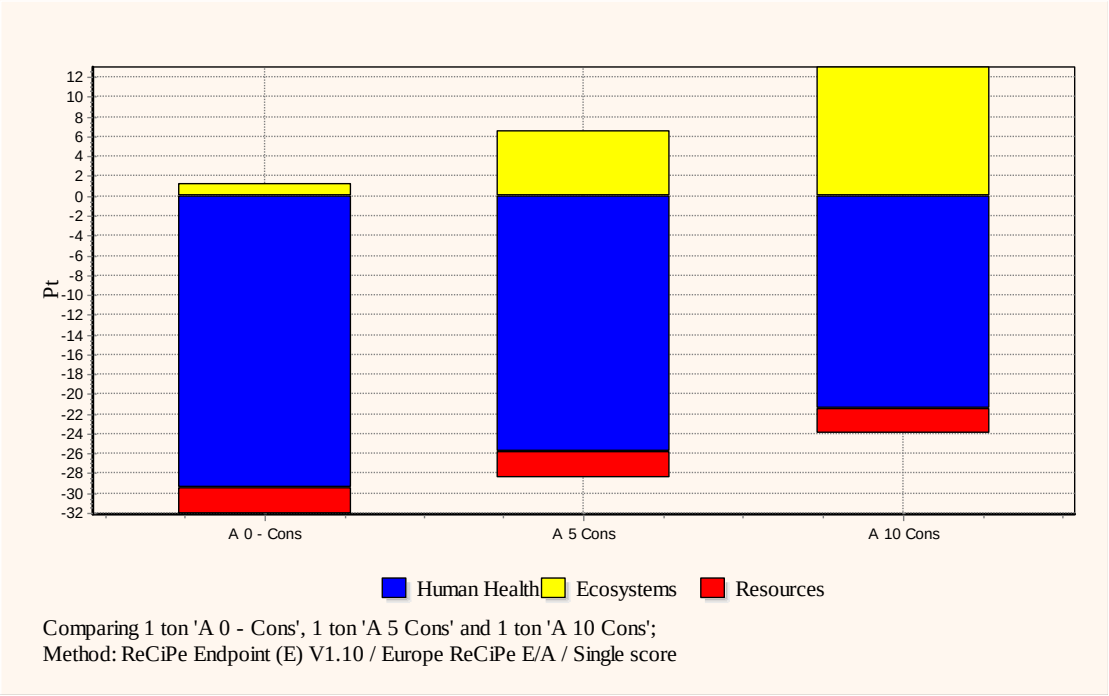

Fig 4-6. Severity of aggregated damage categories according to the ReCiPe methodology for the production of 1 ton compost from bio-waste and application to agricultural site.

4.3.4.2 Comparison of Ag-TiO_2NPs-contaminated compost

Three concentrations of Ag-TiO_2NPs (AT 0, AT 5, AT 10) in compost were used for the calculations. The LCA study was performed using SimaPro 8 software with ecoinvent v3 database. The results calculated using ReCiPe methodology for Europe using H and E perspectives.

4.3.4.2.1 Results calculated using Hierarchist perspective

The single score of the midpoint impact categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost from bio-waste and the application to agricultural site is given in Table 4-10 while the severity of the impact categories is presented in Figure 4-7.

The overall score was calculated to be 7.74 Pt for non-contaminated compost (AT 0) and increased to 12.17 Pt for AT 5 and 17.27 Pt for AT 10. The presence of Ag-TiO_2NPs in compost caused a significant effect in terrestrial eco-toxicity ranging from -0.00777 Pt for A 0 to 2.29 Pt and 4.93 Pt for A 5 and A 10 respectively. The lowest score was calculated for human toxicity category but this beneficial effect was

eliminated due to the presence of Ag-TiO_2NPs as the score is increased from -1.60 Pt for AT 0 to 0.53 Pt and 2.98 Pt for AT 5 and AT 10 respectively. Small differences resulted in scores for marine eco-toxicity and freshwater eco-toxicity due to the presence of NPs in compost.

Table 4-10. Score of midpoint impact categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost and application to agricultural site.

Impact category	Unit	AT 0	AT 5	AT 10
Total	Pt	7.74	12.17	17.27
Climate change Human Health	Pt	5.92	5.92	5.92
Ozone depletion	Pt	-0.00007	-0.00007	-0.00007
Human toxicity	Pt	-1.60	0.53	2.98
Photochemical oxidant formation	Pt	0.00063	0.00063	0.00063
Particulate matter formation	Pt	2.15	2.15	2.15
Ionising radiation	Pt	0.00371	0.00371	0.00371
Climate change Ecosystems	Pt	3.72	3.72	3.72
Terrestrial acidification	Pt	0.03603	0.03603	0.03603
Freshwater eutrophication	Pt	-0.00487	-0.00487	-0.00487
Terrestrial ecotoxicity	Pt	-0.00777	2.29	4.93
Freshwater ecotoxicity	Pt	-0.00484	-0.00428	-0.00379
Marine ecotoxicity	Pt	-0.00097	-0.00090	-0.00086
Agricultural land occupation	Pt	0.14629	0.14629	0.14629
Urban land occupation	Pt	0.04271	0.04271	0.04271
Natural land transformation	Pt	-0.03188	-0.03188	-0.03188
Metal depletion	Pt	-1.37	-1.37	-1.37
Fossil depletion	Pt	-1.24	-1.24	-1.24

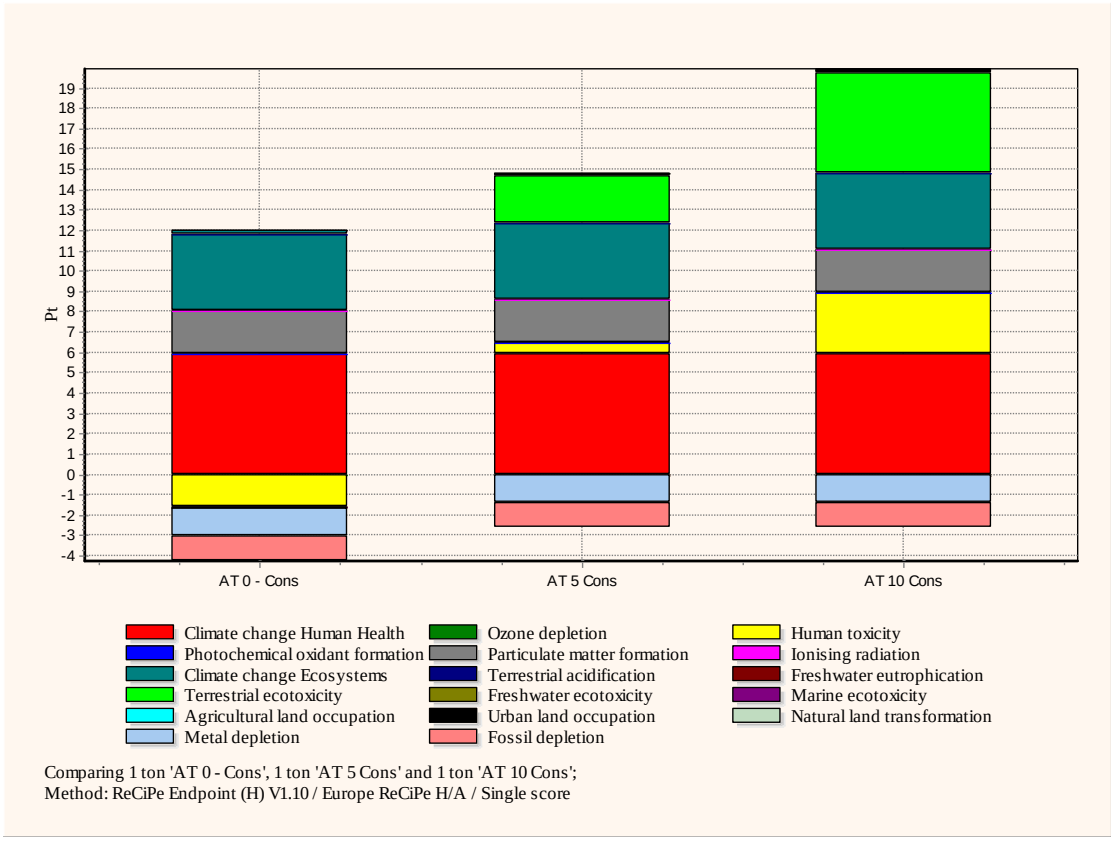

Fig 4-7. Severity of midpoint impact categories according to the ReCiPe methodology, using H perspective, for the production of 1 tn compost and application to agricultural site.

The score of the aggregated damage categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost from bio-waste and the application to agricultural site is given in Table 4-11 while the severity of the aggregated damage categories is presented in Fig 4-8.

The highest score was calculated for human health category for AT 10 (11.05 Pt) compost followed by AT 5 (8.59 Pt) and AT 0 (6.46 Pt). The score of the ecosystems category is increased from 3.90 Pt for AT 0 to 6.19 Pt and 8.84 Pt for AT 5 and AT 10 respectively. The score of resources category was the same for the three types of compost.

Table 4-11. Score of aggregated damage categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost and application to agricultural site.

Damage category	Unit	AT 0	AT 5	AT 10
Total	Pt	7.74	12.17	17.27
Human Health	Pt	6.46	8.59	11.05
Ecosystems	Pt	3.90	6.19	8.84
Resources	Pt	-2.61	-2.61	-2.61

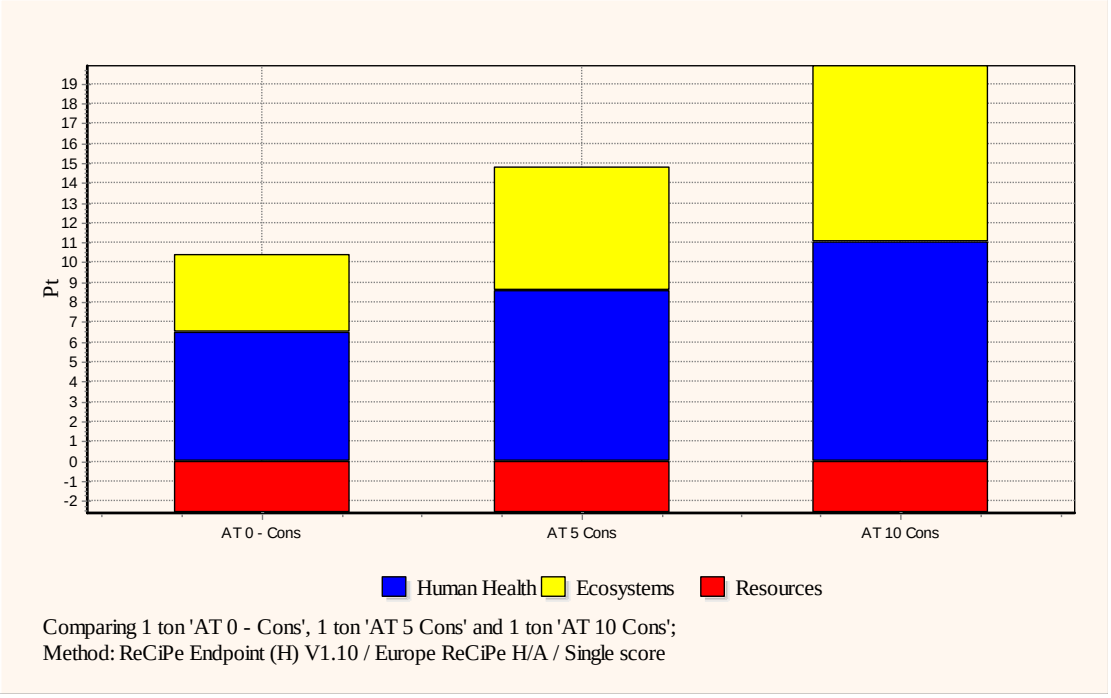

Fig 4-8. Severity of aggregated damage categories according to the ReCiPe methodology, using H perspective, for the production of 1 ton compost and application to agricultural site.

4.3.4.2.2 Results calculated using Egalitarian perspective

The single score of the impact categories according to the ReCiPe methodology, using E perspective, for the production of 1 ton compost from bio-waste and the application to agricultural site is given in Table 4-12 while the severity of the impact categories is presented in Fig 4-9. The overall score was calculated to be -30.85 Pt for non-contaminated compost (AT 0) and increased to -23.07 Pt for AT 5 and -14.11 Pt for AT 10. The presence of AgNPs in compost resulted in a significant effect in terrestrial ecotoxicity as the score increased from -0.05878 Pt for AT 0 to 4.51 Pt and 9.77 Pt for AT 5 and AT 10 respectively. The lower score was calculated for human toxicity category. The beneficial effect to human toxicity is reduced due to the presence of Ag-TiO_2NPs as the score is increased from -30.69 Pt for AT 0 to -27.51 Pt and -23.85 Pt for AT 5 and AT 10 respectively. Small differences resulted in scores for marine eco-toxicity and freshwater eco-toxicity due to the presence of NPs in compost.

Table 4-12. Score of impact categories according to the ReCiPe methodology, using E perspective, for the production of 1 ton compost and application to agricultural site.

Impact category	Unit	AT 0	AT 5	AT 10
Total	Pt	-30.85	-23.07	-14.11
Climate change Human Health	Pt	0.11454	0.11454	0.11454
Ozone depletion	Pt	-0.00004	-0.00004	-0.00004
Human toxicity	Pt	-30.69	-27.51	-23.85
Photochemical oxidant formation	Pt	0.00031	0.00031	0.00031
Particulate matter formation	Pt	1.05	1.05	1.05
Ionising radiation	Pt	0.00182	0.00182	0.00182
Climate change Ecosystems	Pt	0.09626	0.09626	0.09626
Terrestrial acidification	Pt	0.07059	0.07059	0.07059
Freshwater eutrophication	Pt	-0.00320	-0.00320	-0.00320
Terrestrial ecotoxicity	Pt	-0.05878	4.51	9.77
Freshwater ecotoxicity	Pt	-0.00325	-0.00229	-0.00127
Marine ecotoxicity	Pt	-0.66646	-0.63057	-0.59307
Agricultural land occupation	Pt	0.09629	0.09629	0.09629
Urban land occupation	Pt	0.02811	0.02811	0.02811
Natural land transformation	Pt	1.72	1.72	1.72
Metal depletion	Pt	-1.37	-1.37	-1.37
Fossil depletion	Pt	-1.24	-1.24	-1.24

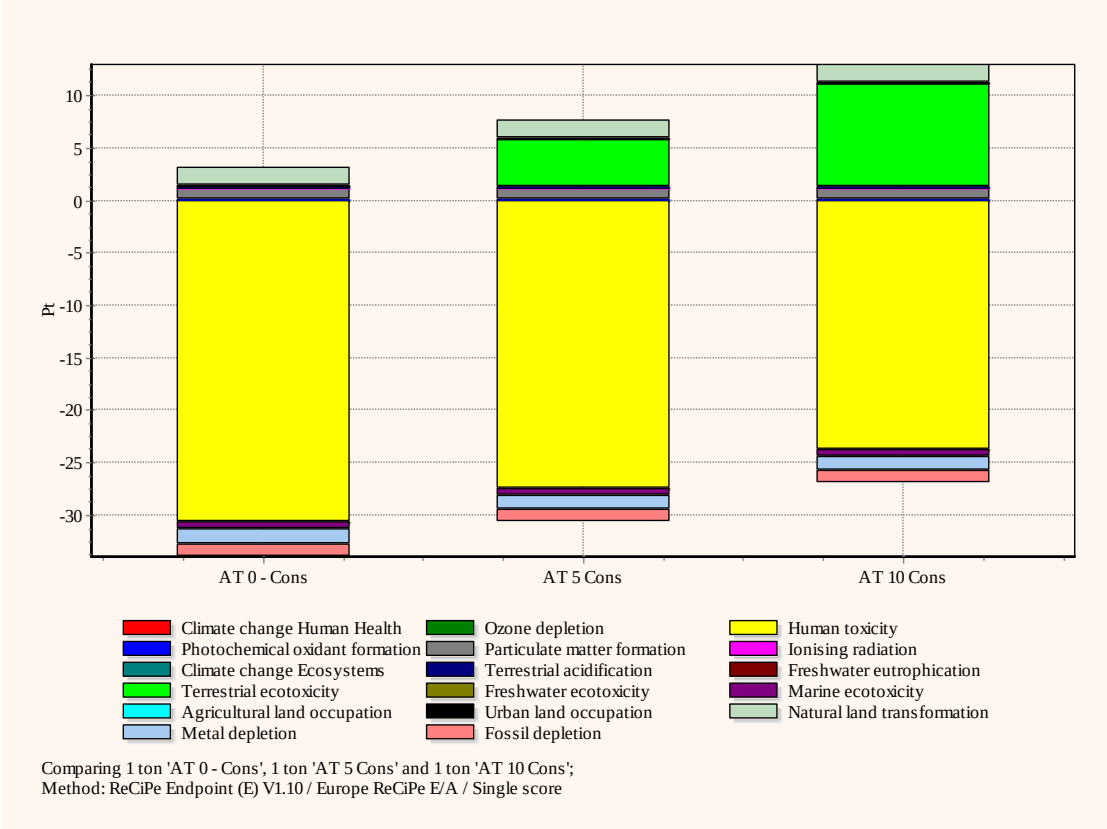

Fig 4-9. Severity of impact categories according to the ReCiPe methodology, using egalitarian perspective, for the production of 1 tn compost from bio-waste and application to agricultural site.

The score of the aggregated damage categories according to the ReCiPe methodology, using E perspective, for the production of 1 ton compost from bio-waste and the application to agricultural site is given in Table 4-13 while the severity of the aggregated damage categories is presented in Fig 4-10.

The highest score was calculated for ecosystems category for AT 10 (11.18 Pt) compost followed by AT 5 (5.89 Pt) and AT 0 (1.28 Pt). The beneficial effect to human health category is reduced due to the presence of AgNPs as the score is increased from -29.52 Pt for AT 0 to -26.34 Pt and -22.68 Pt for AT 5 and AT 10 respectively. The score of resources category was the same for the three types of compost.

Table 4-13. Score of aggregated damage categories according to the ReCiPe methodology for the production of 1 ton compost from bio-waste and application to agricultural site.

Damage category	Unit	AT 0	AT 5	AT 10
Total	Pt	-30.85	-23.07	-14.11
Human Health	Pt	-29.52	-26.34	-22.68
Ecosystems	Pt	1.28	5.89	11.18
Resources	Pt	-2.61	-2.61	-2.61

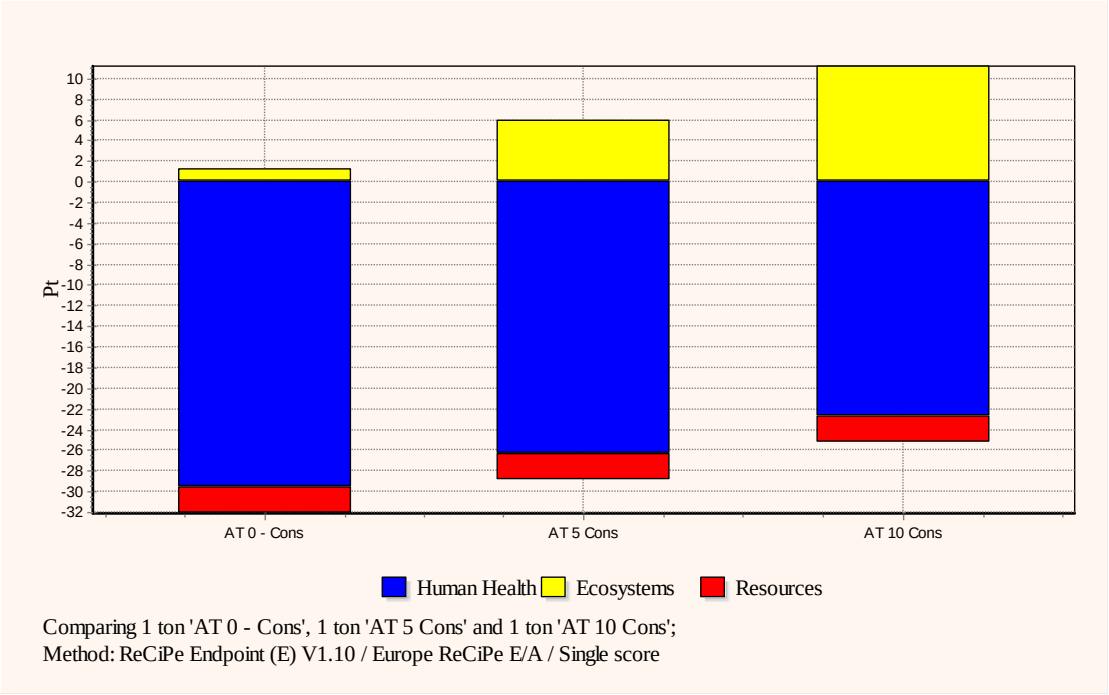

Fig 4-10. Severity of aggregated damage categories according to the ReCiPe methodology for the production of 1 ton compost from bio-waste and application to agricultural site.

4.3.5 Interpretation of results

In comparative LCA studies, the interpretation phase serves to derive robust conclusions and often recommendations (JRC 2010a). In LCA context, the full extent of the likelihood of an effect is accounted using midpoint indicators while the differences in severity are accounted using endpoint indicators. Only emissions that contribute to long-term risk of eco-toxicological effects are accounted without addressing emissions causing short term/acute and local scale effects (JRC 2010b).

The midpoint categories human toxicity, terrestrial ecotoxicity, marine ecotoxicity and freshwater ecotoxicity resulted in different scores when assessing NP-contaminated compost compared to non contaminated compost. Human toxicity and terrestrial ecotoxicity exhibit significant score differences. Small differences resulted in scores for marine eco-toxicity and freshwater eco-toxicity due to the presence of NPs in compost. The characterization factor for human toxicity in ReCiPe method accounts for the environmental persistence (fate) and accumulation in the food chain (exposure) and toxicity (effect) of a chemical (Mark Goedkoop 2009). The exposure routes of metals through bioaccumulation exhibit high uncertainty (De Schryver, Humbert et al. 2013). In hierarchist and egalitarian perspective it is assumed that human exposure occurs via all intake routes (air, drinking water, food) while in individual perspective the routes include only the air and the drinking water (Mark Goedkoop 2009). The calculations of terrestrial fate factors are based on the average concentration of the top 20 cm of the soil only because it is assuming that terrestrial species are exposed to contaminants in the top 20 cm of the soil (Mark Goedkoop 2009). Marine and freshwater toxicity categories show marginal differences due to the low concentration of NPs emitted to surface and ground water compared to the emissions to soil. The calculation of the marine eco-toxicological impacts include both sea and oceanic compartments in egalitarian perspective (Mark Goedkoop 2009).

The endpoints categories ecosystems and human health resulted in score differences. The human health category exhibits the highest score when the calculations were performed using H perspective whereas the ecosystems category exhibited the highest score when using E perspective. The highest score for both impact categories resulted for NP-contaminated compost with the higher concentrations (A 10 and AT 10). The resources category resulted in the same score using both perspectives. The significant score differences resulted in ecosystems and human health categories are mainly attributed to terrestrial ecotoxicity and human toxicity, respectively. It has been

pointed out that the identification and quantification of impacts on human health and ecosystems due to emissions of toxic substances are of central importance in the development of sustainable products and technologies (Rosenbaum, Bachmann et al. 2008). The final ecosystem services have been defined as the components of nature, directly enjoyed, consumed used to yield human well-being (Boyd and Banzhaf 2007). The ecosystems services contribution to human well-being makes human health damage category to be inseparable to ecosystem damage category.

It is very interesting to address the differences in LCA outcome when choosing different perspectives (individualist, hierarchist and egalitarian). Value choices in impact modelling within LCA, such as time horizon, are unavoidable and can have direct implications for LCA outcomes and can result in practical implications on decisions based on the LCA results (De Schryver, Humbert et al. 2013). The different value choices (considering the individualist, hierarchist and egalitarian perspective) use different groups of characterization factors to quantify and aggregate life cycle emissions into damage scores for human health and ecosystems (Mark Goedkoop 2009). The choice of the time horizon and the calculation or exclusion of highly uncertainty effects are considered as the value choices responsible for large differences in LCA results (De Schryver, Humbert et al. 2013). When assessing the performance of individual materials within LCA, it has been reported that the average difference in human health damage scores goes from 1 order of magnitude between the individualist and hierarchist perspectives to 2.5 orders of magnitude between the individualist and egalitarian perspective while can rise up to 5 orders of magnitude depending on the combination of emissions driving the impact of different perspectives (De Schryver, Humbert et al. 2013).

A totally different LCA outcome resulted when using H perspective as compared to the use of E perspective. The score of aggregated damage categories was calculated to be positive when using H perspective and negative when using E perspective. The resulted difference can be attributed mainly to the different time horizon and the highly uncertainty effects calculated for E perspective as compared to H perspective. The negative score that resulted using E perspective implied that the compost production and application of NP-contaminated compost to soil is environmental friendly even for the A 10 and AT 10 composts. The Ag concentration of A 10 and AT 10 was relative high and depending on the application rates may cause significant environmental issues due to their application to soils. In the context of LCA, the

emissions are reported in terms of the mass of each chemical released at each stage of a life cycle to provide a specific amount of a product. Exceeding the current regulatory limits of a chemical at specific locations or points in time is not the focus of a LCA study in contrast with the regulatory assessments of chemical emissions that usually have the goal of evaluating whether there will be an unacceptable risk of a toxicological effect to an individual, sub-population or group of species (Pennington, Margni et al. 2006). It can be concluded that the choice of E perspective failed to reflect significant environmental issues at endpoint categories. In this case, a thorough analysis of midpoint categories results is needed to perform in order to communicate the results and to avoid misunderstanding.

The limitations of the study concerns the Ag and Ti effect calculations and the excluded benefits and emissions due to the compost application to soils. The calculations were based on the Ag and Ti emissions instead of nano-Ag and nano-Ti due to the absence of nano categories in ecoinvent database. The results would be expected to exhibit higher score differences when using nano-Ag because the Ag toxicity depends on the particles size. Significant compost benefits due to the application to soil, such as reduce water use, pesticides and herbicides and reduce requirements of lime and gypsum was difficult to quantified and excluded from the study. Furthermore, risks associated with the emissions to soil, water and air from NP-contaminated compost such as CH_4 emissions and nitrate leaching to surface and ground waters was also difficult to quantify and excluded from the study.

4.3.6 Final remarks

The comparison of NP-contaminated composts resulted in different scores implying that they exhibited different environmental impacts. A totally different LCA outcome resulted when using H perspective as compared to the use of E perspective. Although the performance of compost was calculated to be environmental friendly, when looking at the overall score resulted using E perspective, detailed analysis of the LCA results shall be made as the effects of Ag in terrestrial ecotoxicity and human toxicity were significant and can cause unacceptable risks. The effects of NP-contaminated compost to human health and ecosystems endpoint categories were increased due to the presence of NPs. The risks were associated with terrestrial ecotoxicity and human toxicity midpoint categories and were mainly attributed to the accumulation of Ag to soils.

CHAPTER 5: GENERAL DISCUSSION

The research regarding the impact of NPs on waste treatment technologies and the NPs fate in the environment is on its early stages. As far as the author is aware, only one study has been published on the impact of AgNPs on composting process while a couple of studies have been published on the impact of AgNPs on anaerobic digestion. The impact of other types of NPs on composting has not been investigated yet. Regarding the published study of AgNPs on composting process, the researchers investigated the impact of PVP-coated AgNPs on composting of MSW at a concentration of 2 mg Ag / kg on waste and focused on the impact NPs on bacterial diversity (Gitipour, El Badawy et al. 2013). The results of this study are in congruence with the results of the published study regarding the impact of AgNPs on composting process. Regarding similar range of NPs concentrations on waste, both studies suggest that the presence of AgNPs on waste did not significantly affect the functional performance of composting process. The results based on Gitipour et *al.* (2013) showed that the overall bacterial community changed in response to the NPs presence on waste but these changes did not affect the process due to potential bacterial functional redundancy within the compost samples. The investigation of the impact of NPs on bacterial diversity was outside the scope of this thesis.

The first part of this research was laboratory based and focused on testing the thesis hypothesis. The hypothesis of the thesis has been stated as follow: *"As AgNP have antibacterial properties, it is therefore expected that they will have an impact on the composting process. Their subsequent release into the environment through the application of compost to the soil could either induce toxicity to plants, accumulate in the soils and/or leach into groundwater systems"*. The impact of NPs on composting process was investigated using commercial AgNPs and Ag-TiO_2NPs at different concentrations of NPs on waste. The research findings indicate that NP presence on waste did not induce any toxicity to composting system within the range of the concentrations tested. The process proceeded with the formation of HS and this is an indication of the compost stability. The humification progress is closely related with compost stability because HS are inherently difficult for microbes to decompose and this property enhances the carbon sequestration. Higher carbon mineralization rates resulted for NP-contaminated compost with the higher NP concentrations. This result indicates that NPs presence in waste may have an impact on humification progress.

The results exhibit no significant differences in HA and FA concentrations of mature samples. The concentration of humins was not investigated due to the absence of a standard quantification method. Humins constitutes the main fraction of HS in terms of total carbon present in HS. A difference in humin formation rates may have a significant impact in carbon mineralization rates and this may explain the research findings which indicate higher mineralization rates due to the NPs presence. The set of experiments using Ag-TiO_2NPs resulted in higher mineralization rates compared to the one using AgNPs. This may indicate the additional impact of TiO_2-NPs in the process. The above research findings indicate that the presence of NPs in waste streams may have an impact on composting process but this impact does not affect the functionality of the process.

The EEM fluorescence spectroscopy was successfully used to characterize and quantify the HA and FA present in compost extracts. Due to the complex nature of the samples, the effect of interferences and overlapping peaks was minimised by introducing a separation step of HA and FA. While all analyses conducted in triplicates corroborated the location and intensity of the peaks associated to HA and FA, it is possible that the separation step may have had an effect on the location and intensity of the mentioned peaks. It is therefore recommended, that in future studies, a statistical analysis of 3D data using parafac analysis is conducted.

The experimental results consider only the impact of AgNPs and Ag-TiO_2NPs on composting process and their fate on the environment. The presence of different types of NPs in waste could provide a better simulation of the real conditions but this was outside the scope of this study. Further research is required to investigate the impact of other types of NPs on composting processes in conjunction with AgNPs and/or Ag-TiO_2NPs. Different types of NPs may result in different impacts (*e.g.*, positive or negative; synergetic or antagonic) on composting processes. A very complex system would result which needs to be investigated to have a better idea of the impact of NP on composting process.

The toxicity to plants due to NP-contaminated compost application to soil was tested using a cress germination test. The germination indexes indicate no phytotoxic effects even at high concentrations of AgNPs and Ag-TiO_2NPs in compost samples.

The accumulation of NPs to soils and/or leaching to groundwater systems was investigated using a column leaching experiment. The leaching results indicate that NPs mainly accumulate to soil following application of NP-contaminated compost to

soils, as only a small percentage of NPs was determined in HA and FA fractions. The NPs accumulated to soil may subsequently leach to surface (i.e., runoff) or to ground waters. The particle size of the NPs present in HA and FA was detected to be in the nano region and this may have further environmental implications as NPs exhibit higher mobility compared to larger clusters of particles and furthermore their toxicity effects depend on their size.

It should be highlighted that, when measuring nanoparticles in complex environmental samples, it is not only necessary to generate data on concentrations but information will also be required on the size distribution and properties of the particles, as aforementioned. At present there is no single technique that can provide all this information, so a range of analytical techniques is required. Moreover, an accurate prediction of nanoparticles in complex environmental samples is not possible at present based on available methods as they do not fulfil all data requirements.

In fact, many analytical tools are theoretically suitable for the characterization of nanoparticles (*e.g.,* electron microscopy, dynamic light scattering, field flow fractionation techniques), but only a few of these are applicable to the analysis of complex environmental samples. Requirements for analysis of commercial nanoparticles in compost matrices, will differ greatly from their analysis in pure or neutral media (*e.g.*, distilled water). It should be also highlighted that many techniques are destructive or, if not, application of some sample preparation methods can lead to artifacts, as previously discussed. It is therefore recommended that in future studies, environmental samples of diverse elemental compositions containing more than one type of nanoparticle would be analysed. It is envisaged that this effort will progressively allow the development of prediction techniques and models.

The hypothesis of the thesis was rejected in terms of the possible impact of NPs on composting process due to their toxicity and the possible toxicity that may induce to plants through the NP-contaminated compost application to soil. In terms of NPs release into the environment through the application of NP-contaminated compost to soil and their subsequent accumulation to soils and/or leaching into groundwater systems the hypothesis of the thesis was confirmed.

The second part of this research focused on the investigation of the possible environmental impacts due to the NP-contaminated compost production and application to soil through a comparative LCA study. The avoided fertilizer production and carbon sequestration were accounted as the main benefits of compost

application to soil whereas emissions include the NPs emissions to soil and water. Other compost benefits of the same significance were excluded from this study as they were difficult to quantify. Also, emissions due to the application of compost to the soil, such as CH_4 emissions and nitrate leaching to surface and ground waters were also difficult to quantify and therefore excluded from this study.

A consequential LCA study was performed using the ReCiPe method for Europe with hierarchist and egalitarian perspective. Major differences in LCA scores resulted between hierarchist and egalitarian perspective and this can be attributed more to the fact that egalitarian perspective is a long term perspective whereby a minimum of scientific proof justifies inclusion. The choice of egalitarian perspective failed to reflect significant environmental issues at endpoint categories. The total score was negative indicating that the product is environmental friendly but looking at midpoint categories the impact on terrestrial ecotoxicity and human health categories was significant.

The results were analysed using the calculations based on the hierarchist perspective. The score of the endpoint categories "human health" and "ecosystems" is increased due to the presence of NPs in compost for both composts contaminated with AgNPs and $AgTiO_2$-NPs. The midpoint categories that exhibit the higher increase due to the NPs presence in compost are the "terrestrial ecotoxicity" and "human toxicity" and this can be attributed to the accumulation of NPs to soils. The midpoint categories "marine ecotoxicity" and "freshwater ecotoxicity" exhibit small differences due to the leaching of NPs to surface and ground waters. The scores exhibit no significant differences between AgNPs and Ag-TiO_2NPs contaminated compost. This may indicate that the contribution of Ti emissions to "terrestrial ecotoxicity" and "human toxicity" categories is negligible. In conclusion, the LCA study resulted in major environmental impacts of NP-contaminated compost mainly attributed to the use of compost as a fertilizer and the subsequent emissions of NPs to soil and water.

The assessment of uncertainty was not carried out in the presented LCA analysis. This is due to the absence of Monte Carlo tool in the LCA software package purchased. In stipe of that, the use of two different perspectives in LCIA phase (Egalitarian and Hierarchist) resulted in totally different outcomes. This difference can be attributed mainly to the different time horizon and the highly uncertainty effects calculated for E perspective as compared to H perspective. In any case it should be highlighted that the comparison of the two different perspectives LCA outcomes resulted in useful

conclusions, such as the failure of the Egalitarian perspective to address significant environmental issues.

The overall results indicate that the presence of NPs in waste streams may have an impact in sustainable land management and water resources through the application of NP-contaminated compost to soil. Non-hazardous waste (manures, biosolids, composts, wastewater effluents, etc) have been applied to land for centuries as a means of disposal and to improve the soil via the recycling of nutrients and the addition of organic matter. Compost produced from source-separated organic residues may be a source of contamination when applied to the soil. The contamination of arable land aggravates the quality of soils and crops and can affect food quality and human health.

The issues of soil and water contamination are addressed from the environmental, social and economic point of view.

In terms of environment, soil and water pollution endanger life and species and create a variety of health hazards. The soil functions as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water quality, and promote plant and animal health (Doran and Zeiss 2000). The presence of toxic elements in soil affects the soils health. The soil contamination may have a significant impact in soil microbial activity depending on the contaminants and their concentration. The soil and water contamination may cause toxicity to plants and animals depending on element concentration and availability. In general, soil and water pollution results in deterioration of the environment's quality.

In terms of society, the soil and water contamination endanger the human health and safety. The persistent contaminants accumulate in living organisms and tend to increase up the food chain with greatest concentrations in high-end predators. The main exposure routes for humans include ingestion and inhalation. The infiltration of soil contamination into groundwater used for human consumption can pose significant risks to human health. The concentration of a toxic element in aquatic biota may be many times higher than that in the surrounding water due to the bioaccumulation of the element throughout an organisms lifetime and the biomagnification of the contaminant up the food chain.

In terms of economy, soil and water pollution have direct and indirect costs. Direct costs include: the amount of money spend on site characterization, risk management and remediation of contaminated soil and water; the possible restrictions to the use of

land for agriculture and the possible restricted access to safe drinking water. Indirect costs result from land users responses to soil contamination such as abandoning cultivated areas, loss of capital and consequently unemployment.

The three pillars of sustainability, environmental, economic and social are affected due to the presence of NPs on waste streams and the application of NP-contaminated compost to the soil. The NP-contaminated compost becomes less sustainable product as the concentration of NPs increases. Consequently, composting process may become a less sustainable process in future due to the increased concentration of NPs on waste. Nowadays, composting is recognised as one of the most sustainable waste management technologies so the case that compost sustainability decreases due to the presence of NPs on waste may lead policy makers to reconsider about recommended composting and shift to more sustainable solutions in the future.

CHAPTER 6: CONCLUSIONS

The aim of this thesis was to assess the impact of Ag-TiO_2NPs and AgNPs on the in-vessel composting of biodegradable MSW, the NPs fate and toxicity in the environment and the positive and negative environmental impacts of compost application to soils due to the presence of AgNPs and Ag-TiO_2NPs in waste streams. The impact of NPs in composting was assessed by monitoring the temporal dynamics of organic matter (OM) using Excitation Emission Matrix (EEM) fluorescence spectroscopy. The NPs fate in the environment was investigated using a column leaching experiment while the phytotoxicity of NP-contaminated compost was assessed using a seed germination bioassay. The possible environmental impacts due to the application of compost contaminated with NPs to soils were investigated by conducting a comparative LCA study.

The main conclusions of this study are therefore as follows:

- The presence of 2% NaN_3 in biodegradable MSW inhibited effectively the microbial activity during the first week of composting. The microbial population activated following the second week of composting but the decomposition rate was so low that did not result in the formation of HS following 21 days of composting.
- Both treatments, using Ag-TiO_2-NPs and AgNPs, did not show any inhibition of the decomposition process for all the tested concentrations and the EEM peaks shifted towards the HS region. Higher inorganic carbon removal resulted for NP-contaminated compost with the higher NP concentrations. This may indicate that the formation of humins was higher for non-contaminated compost and decreased as the NP concentration in waste increased.
- The shift of the peaks towards the HS region during composting for all the treatments indicated that NPs did not have an effect in humification and therefore in compost stability. The weight loss percentage, on dry weight, was *ca.* 40% during composting of all treatments which implies the good performance of the method especially when comparing with NaN_3 treatment where the weight loss percentage was 2.88%.
- The use of EEM fluorescence spectroscopy analysis required a first separation of HA and FA step, because their peaks interfere and overlap in EEM spectra

and this can result in wrong estimation of the peaks location and intensity if the sample was analysed as a mixture.

- The NP-contaminated compost exhibited no phytotoxic effects to the cress seed germination tests even at high concentrations of AgNPs and Ag-TiO_2NPs in compost.
- The leaching results suggested that only a low percentage of the total NPs (in weight) in compost, up to *ca.* 5% for Ag and up to *ca.* 15% for Ti, leached to the environment. This indicated that NPs mainly accumulate in soils following application of NP-contaminated compost. It is suggested that long term studies should be conducted to corroborate that these results remain over longer time and also contrasting meteorological conditions (e.g., flooding events).
- The LCA study indicated that the effects of NP-contaminated compost to human health and ecosystems endpoint categories increased due to the presence of NPs. The risks were associated with terrestrial ecotoxicity and human toxicity midpoint categories and were mainly attributed to the accumulation of Ag to soils.
- A detailed analysis of the LCA results shall be made in both midpoint and endpoints impact categories when the calculations are based on the egalitarian perspective. In this study, the choice of egalitarian perspective failed to reflect significant environmental issues at endpoint categories.

Finally, while the results of this research indicated that the presence of AgNPs and Ag-TiO_2NPs in waste did not significantly affect the functional performance of composting processes, the subsequent application of compost contaminated with these NPs to soils may pose risks to the environment and human health. The risks are mainly associated with the emission of AgNPs to soil and waters and depend mainly on the concentration levels of Ag. Nowadays, the concentration levels of AgNPs in compost produced from MSW are unknown. The absence of standard methods to identify Ag-NPs in complex waste and compost matrices, and to distinguish them from the other forms of Ag that might be present in these matrices, make their quantification very difficult, if not impossible at present. To overcome this limitation, an estimation could be made by measuring the total Ag content as sources of Ag in MSW are limited. Additionally, the absence of European legislation establishing

statutory limits of Ag in biosolids for land application is of current concern. Based on the results of this research and current analytical and regulatory limitations, this study recommends: (i) that the concentration of Ag in compost is monitored (as the AgNPs production has rapidly increased worldwide and high concentration levels of AgNPs will undoubtedly increase in the future); (ii) that further research is conducted to investigate the impact of other types of NPs on composting process in conjunction with AgNPs or Ag-TiO_2NPs as well as associated impacts that the resulting compost may have on the environment and human health.

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APPENDIX A: FLUORESCENCE ANALYSIS

A.1.1 The effect of pH in fluorescence analysis

The effect of pH in fluorescence analysis was determined using HA standards obtained from IHSS. 5 mg/L Pahokee Peat HA standard solutions were prepared at different pH values (4.10, 6.01, 8.15 and 9.10). The pH was adjusted using 0.1N HCl and 0.1 N NaOH. The EEM spectra are presented in Figure A-1. The shape of the peaks didn't exhibit any distinct differences. The peak intensities are given in Table A-1. The solutions with pH 6.01 and 8.15 resulted in the higher intensity values for both peaks. This indicate that the pH range around the neutral region is the optimum to quantify HA using EEM fluorescence spectroscopy.

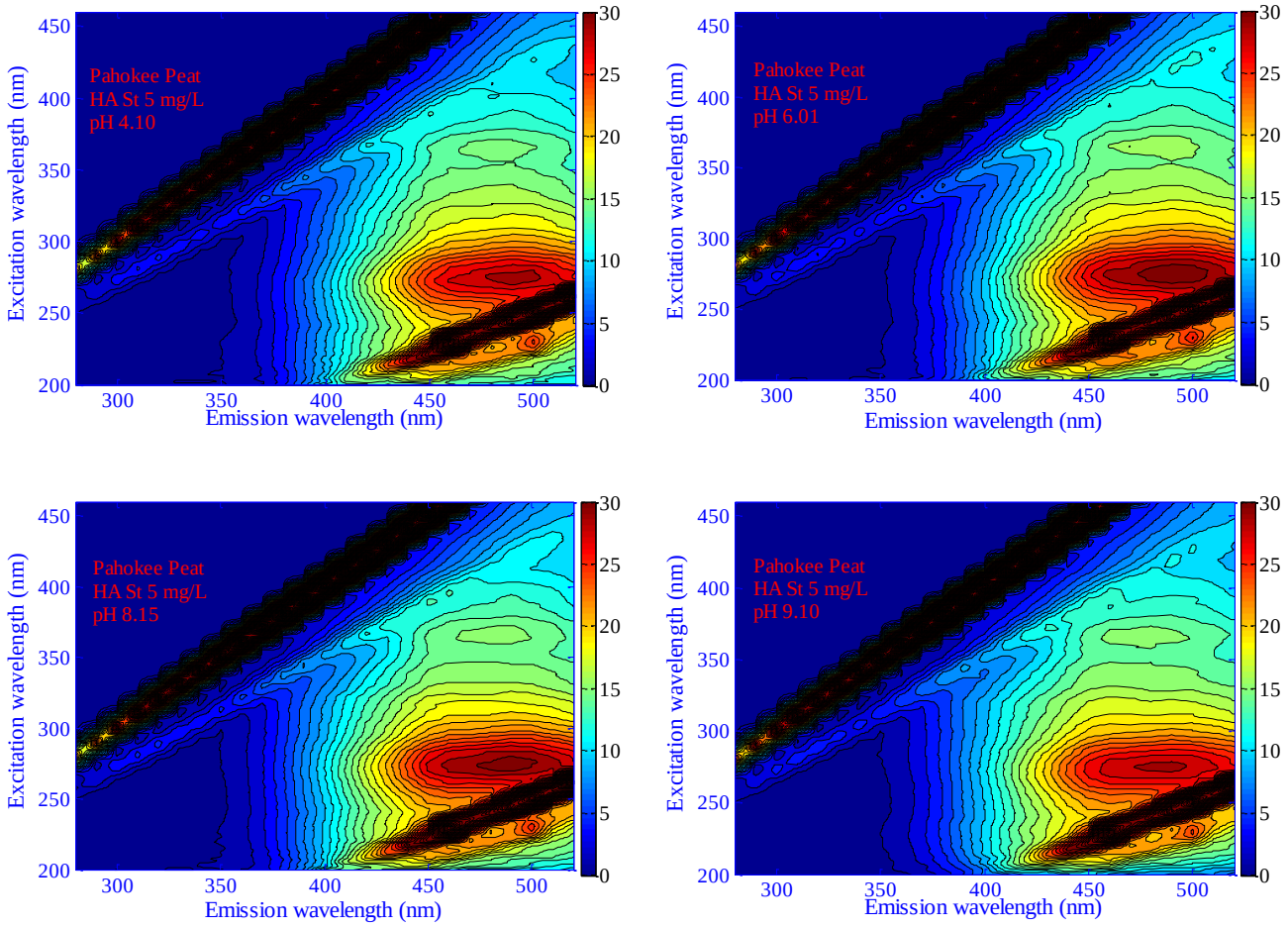

Fig A-1. EEM spectra of Pahokee Peat HA standard at different pH values.

Table A-1. Peak intensities for Ex/Em 275/480 nm/nm and 365/480 nm/nm.

	Ex/Em 275/480	Ex/Em 365/480
pH 4.10	28.9881	15.3853
pH 6.01	30.6079	15.9117
pH 8.15	29.954	15.7327
pH 9.10	28.9338	15.2689

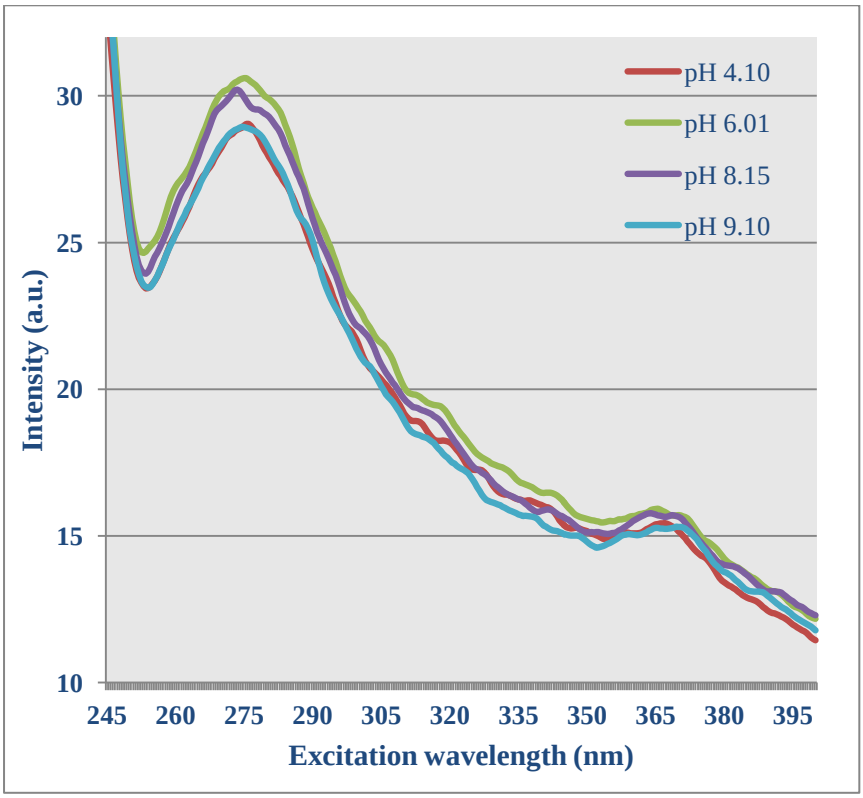

Fig A-2. Fluorescence intensity (a.u.) at emission wavelength 480 nm of Pahokee Peat HA standard at different pH values.

A.1.2 Characterization of HA and FA peaks in a HS solution

HA and FA peaks in a HS solution were characterized using HA and FA standards obtained from IHSS. Three different solutions were prepared: (a) 2 mg/L Pahokee Peat HA standard, (b) 2 mg/L Pahokee Peat FA standard and (c) a HS standard sample containing 2 mg/L Pahokee Peat FA standard and 2 mg/L Pahokee Peat HA standard. The EEM spectra for the solutions are presented in Figure A-3.

HA and FAs are characterized as polydispersed macromolecules and thereby exhibit a range of molecular weights from as low as a few hundred for FA to as much as several hundred thousand for HA. This indicate that the characterization of HA and FA using EEM fluorescence spectroscopy will result in more than one peaks because the location of the peaks differs for different molecular weights. This is obvious from the HA and FA standard analysis spectra (see Figure A-2) and also in the spectra provided from IHSS (see Figure 2-7).

A useful definition for HA and FA, in terms of fluorescence properties, can be derived from the results as follow:

HAs and FAs can be defined, in terms of their fluorescence properties, as substances that exhibit peaks with emissions wavelengths up to 450 nm and higher than 460 nm respectively.

The FA standard exhibits 2 main peaks at Ex/Em 268/450 and 320/450 nm/nm. The HA standard exhibits 2 main peaks at Ex/Em 275/460 and 365/460 nm/nm. It is well known that HA standard exhibit one more peak at Ex/Em 460/530 but this peak is difficult to detect in so diluted solutions. The HS standard exhibits 3 main peaks at Ex/Em of 275/460, 320/450 and 365/460 nm/nm. The intensities of the detected peaks are given in Table A-2. The peak intensities detected in the HS are higher compared to the peak intensities of the pure FA solution and much higher compared to the peak intensities of the pure HA standard solutions. Also, the peaks with Ex/Em 268/450 and 275/460 nm/nm overlapped resulting in one peak at Ex/Em 275/460 nm/nm. The results indicate that it is almost impossible to qualify and quantify FA and HA in a HS solution because of the resulting overlapping and the interference between FA and HA which results in overlapping and wrong estimation of the peaks intensity. One solution is to separate HA and FA and detect the two substances separately. Another solution is to use the PARAFAC modelling to decompose the excitation emission matrix resulting in separated peaks for HA and FA.

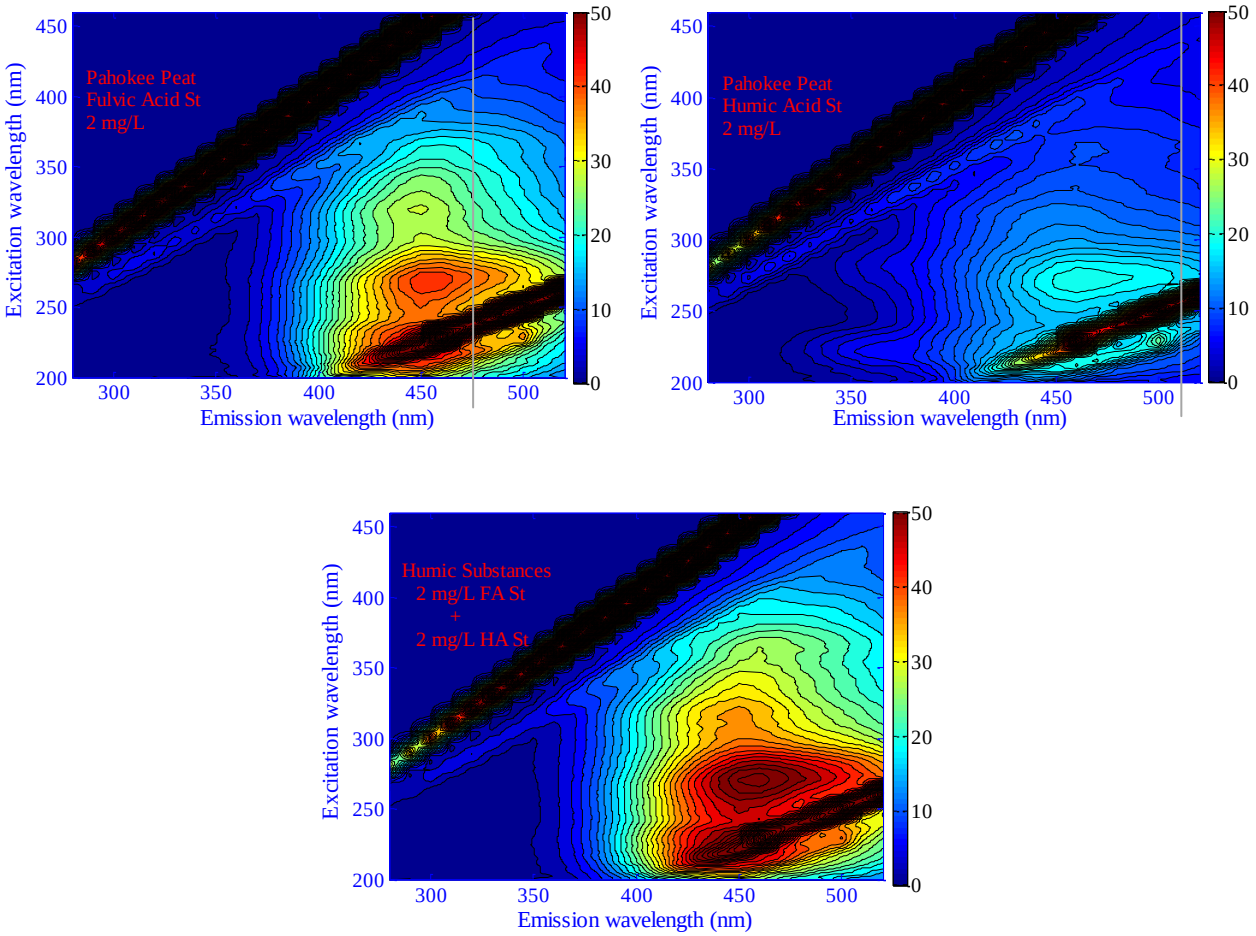

Fig A-3. EEM spectra for HA, FA and HS standard solutions.

Table A-2. Intensity of peaks detected in HA, FA and HS standard solutions.

	Intensity (a.u.)	**Intensity (a.u.)**	**Intensity (a.u.)**	**Intensity (a.u.)**
	Ex/Em 268/450	Ex/Em 320/450	Ex/Em 275/460	Ex/Em 365/460
FA St	42.83	29.56	-	-
HA St	-	-	19.95	9.61
HS	53.77	36.30	54.03	27.97

A.1.3 Inner Filter Effect

The inner filter effect was estimated using EEM spectra of concentrated solutions and the results obtained from the quantification HA and FA standards. Three solutions of Leonardite HA standard were prepared at concentrations of 5, 20 and 50 mg/L. The EEM spectra are presented in Figure A-4.

At concentration 20 mg/L, the peak with Ex/Em 460-470/530-540 nm/nm is clearly appeared compared to the concentration of 5 mg/L that appears as a shoulder at Ex/Em 460/530 nm/nm. At 50 mg/L, all the peaks have shifted to higher emission

wavelengths. The Rayleith scatter appeared in the lower excitation wavelengths, is clear for 5 mg/L but it becomes narrow for 20 mg/L and disappears for 50 mg/L.

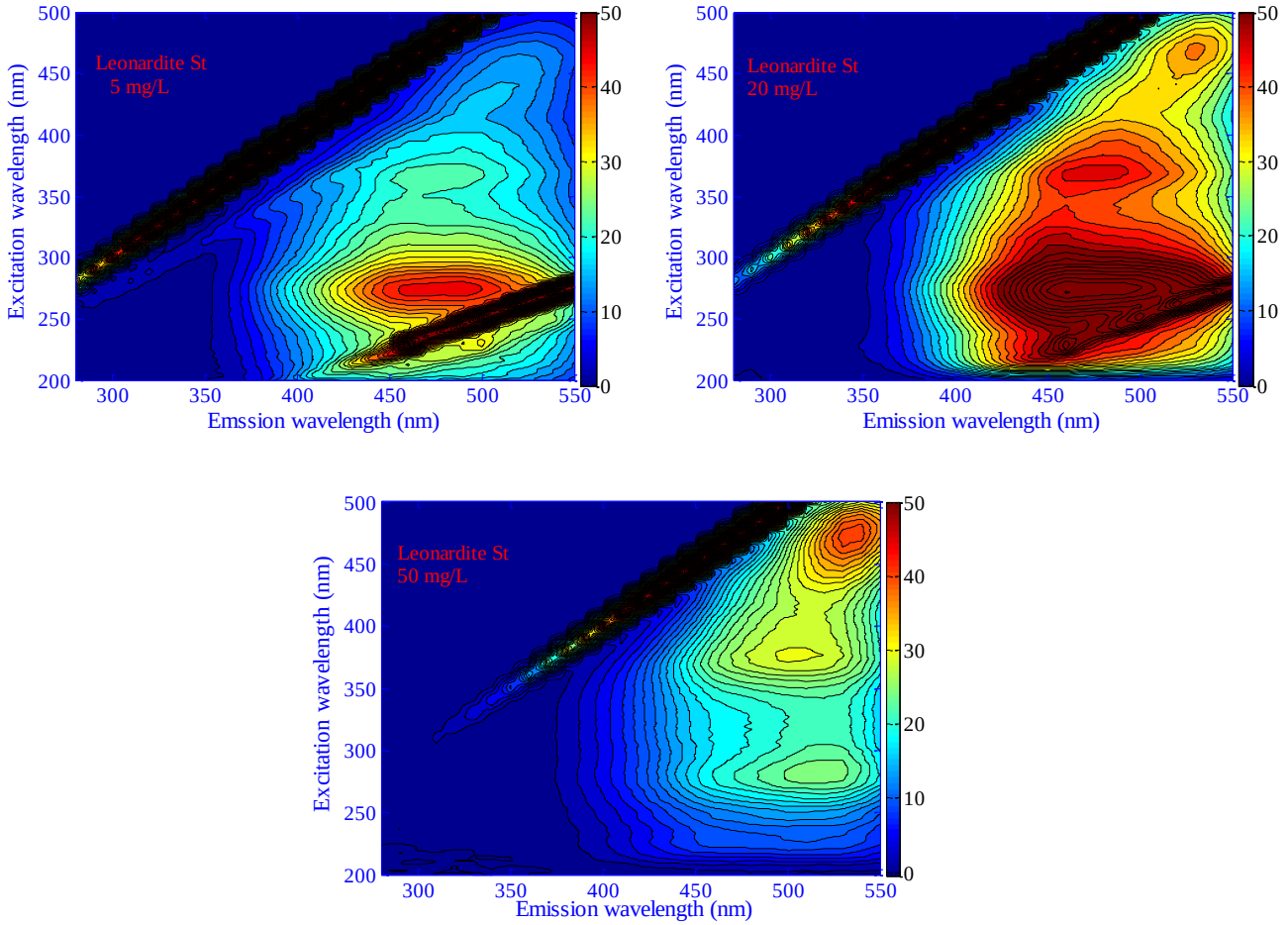

Fig A-4. EEM spectra for Leonardite HA standard at 5, 20 and 50 mg/L.

The quantification of Pahokee Peat FA standard showed that the peak intensity at Ex/Em 268/450 nm/nm is quenching for concentrations higher than 5 mg/L. The quantification of Leonardite HA standard showed that the peak intensities at Ex/Em 275/460 nm/nm and 365/460 nm/nm are quenching for concentrations higher than 3 mg/L for both peaks. The quantification of Pahokee Peat HA standard showed that the peak intensities at Ex/Em 275/460 nm/nm and 365/460 nm/nm are quenching for concentrations higher than 4 mg/L and 5 mg/L respectively. The different concentration limits for intensity quenching between the HA standards resulted due to the higher carbon content of Leonardite HA standard compared to the Pahokee Peat HA standard (see Table 2-6).

A.1.4 HA and FA quantification

A series of HA standards were prepared using Leonardite HA standard and Pahokee Peat HA standard to produce the HA calibration curve. The intensity was measured for peaks with Ex/Em 275/460 nm/nm and 365/460 nm/nm for both standards. The results are given in Table A-3 and Table A-4. Calibration curves of Leonardite HA standards for peaks with Ex/Em 275/460 nm/nm and 365/460 nm/nm are given in Figure A-4 and Figure A-5 respectively. Calibration curves of Pahokee Peat HA standards for peaks with Ex/Em 275/460 nm/nm and 365/460 nm/nm are given in Figure A-6 and Figure A-7 respectively.

Table A-3. Peak intensity, pH and absorption at λ=254nm values for Leonardite HA standard solutions.

	Conc.(mg/L)	Int. (a.u.) Ex/Em 275/460	Int. (a.u.) Ex/Em 365/460	pH	UV-254
St 1	1	11.749	4.967	7.9	0.045
St 2	1.5	20.368	8.681	8.05	0.092
St 3	2	27.413	12.497	8.2	0.136
St 4	2.5	34.067	16.268	8.15	0.182
St 5	3	40.316	20.182	7.65	0.228
St 6	4	45.132	23.165	8.15	0.274
St 7	5	50.219	25.999	7.9	0.32
St 8	6	52.176	29.604	8.15	0.369
St 9	7	55.563	29.97	7.5	0.411
St 10	8	57.382	29.766	8.2	0.458

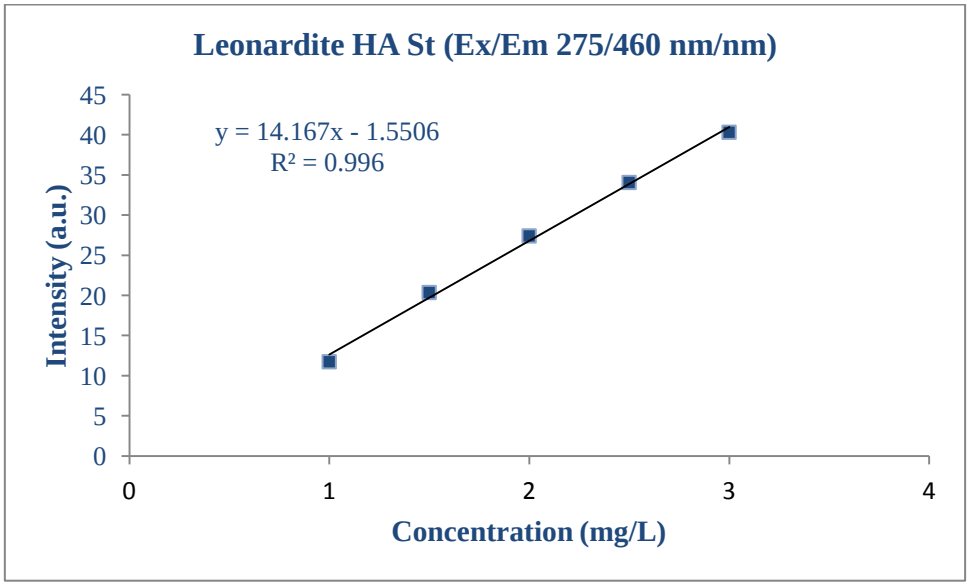

Fig A-5. Calibration curve for peak with Ex/Em 275/460 nm/nm of Leonardite HA standards.

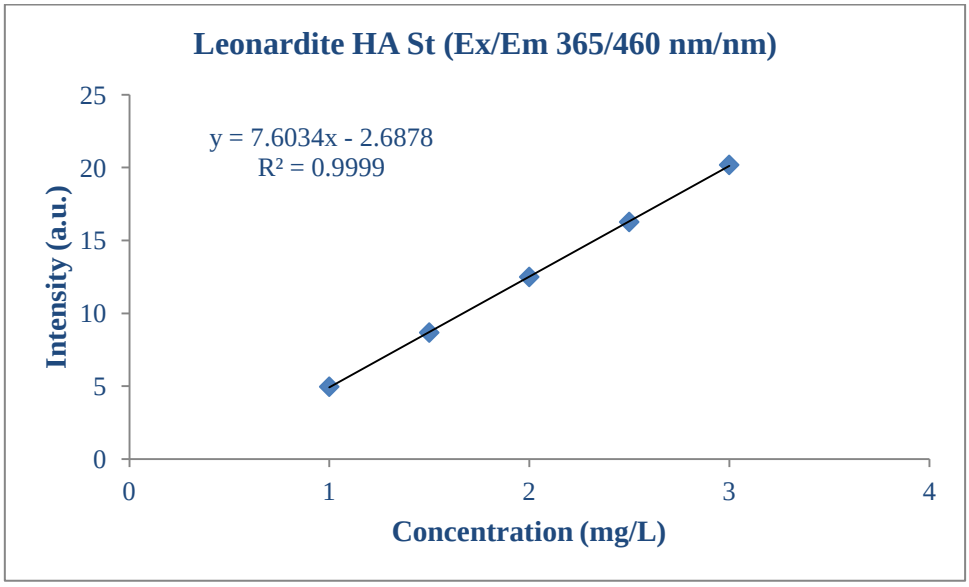

Fig A-6. Calibration curve for peak with Ex/Em 365/460 nm/nm of Leonardite HA standards.

Table A-4. Peak intensity, pH and absorption at λ=254 nm values for Pahokee Peat HA standard solutions.

	Conc.(mg/L)	Int. (a.u.) Ex/Em 275/460	Int. (a.u.) Ex/Em 365/460	pH	UV-254
St 1	1	14.136	6.132	8.1	0.07
St 2	1.5	19.647	9.698	7.96	0.102
St 3	2	24.333	12.399	8.13	0.137
St 4	2.5	28.572	14.713	8.15	0.173
St 5	3	32.739	16.335	7.97	0.210
St 6	3.5	35.724	18.626	8.05	0.242
St 7	4	38.11	20.954	7.98	0.276
St 8	4.5	39.991	22.861	7.65	0.312
St 9	5	43.429	24.969	8.17	0.346

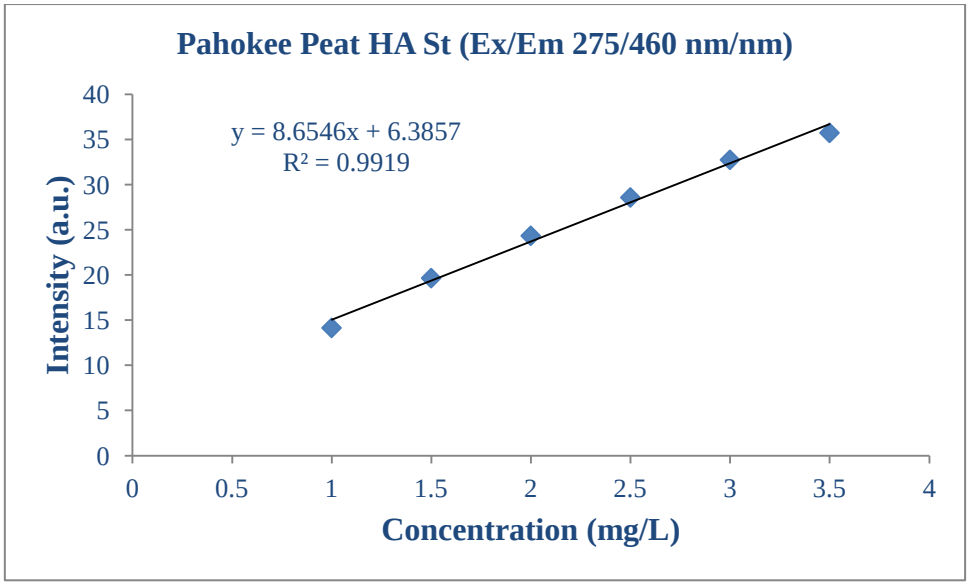

Fig A-7. Calibration curve for peak with Ex/Em 275/460 nm/nm of Pahokee Peat HA standards.

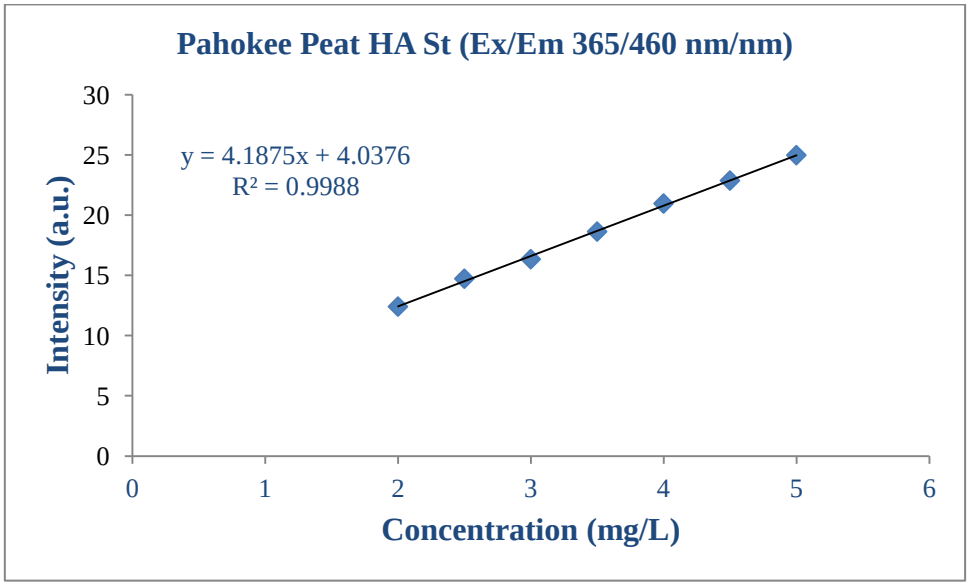

Fig A-8. Calibration curve for peak with Ex/Em 365nm/460nm of Pahokee Peat HA standards.

The ratio of the intensities for the peaks with Ex/Em 365/460 nm/nm and 275/460 nm/nm was calculated to be close to 0.5. A simple definition and an indicator which can be used as a maturity index can be derived from the quantification results, as follow:

A compost can be characterized as mature, in terms of its fluorescence properties, when the peaks of HA have emission wavelength of 460 nm or higher at excitation wavelength of 275 nm and 365 nm.

A compost can be characterized as mature when the ratio of the intensities for the peaks with Ex/Em 365/460-490 nm/nm and 275/460-490 nm/nm are close to 0.5 when diluted the sample to have an absorbance 0.2Å at 254 nm.

For the FA calibration curve, a series of FA standards were produced using Pahokee Peat FA standard. The intensity was measured for Ex/Em 268/450 nm/nm. The results are given in Table A-4. The calibration curve is presented at Figure A-8.

Table A-4. Peak intensity, pH and absorption at λ=254nm values for Pahokee Peat FA standard solutions.

	FA St	268/450		
	Conc.(mg/L)	Int	pH	UV-254
St 1	1	20.804	8.01	0.027
St 2	1.5	31.087	8.15	0.042
St 3	2	39.297	8.23	0.056
St 4	2.5	47.703	8.2	0.075
St 5	3	55.406	8.25	0.088
St 6	4	70.653	8.42	0.119
St 7	5	86.403	8.4	0.152

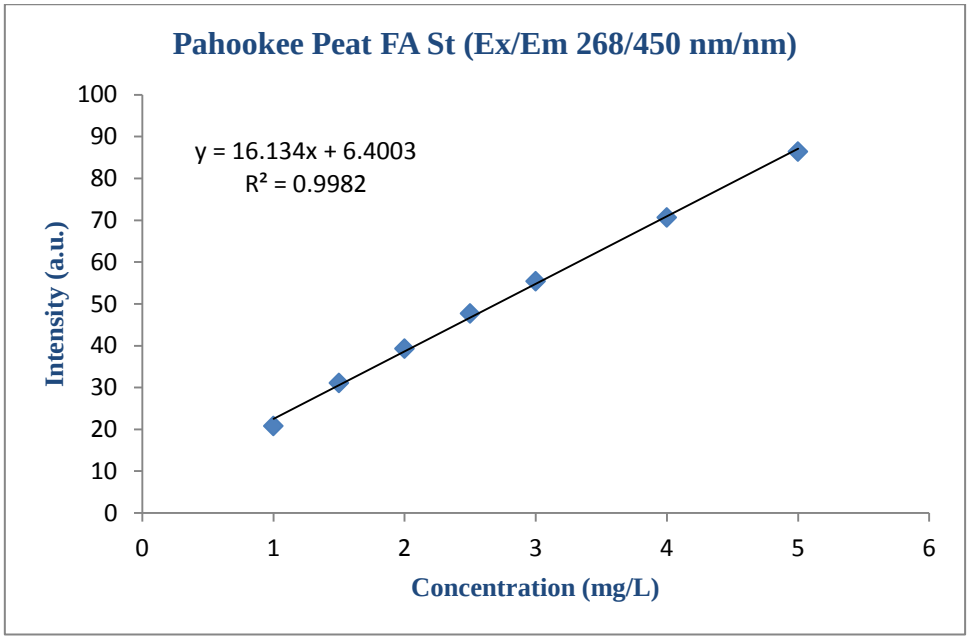

Fig A-9. Calibration curve for peak with Ex/Em 268/450 nm/nm of Pahokee Peat FA standard.

A.1.5 Results for HA quantification of mature samples

The samples diluted to 1:50 prior to analysis. Intensity values of peak with Ex/Em 365/460 and calculated HA concentrations using the Leonardite HA standard are given in Table A-5.

The HA concentrations were calculated by multiplying the concentrations obtained for the measured intensities with the dilution factor. The average values for the different treatments are given in Table A-6. The concentration normalized to the carbon content of the HA standard based on the carbon content of mature samples. Normalized concentrations are given in Table A-7. Furthermore the concentrations converted to mg/g of compost. The results are given in Table 3-9 and Table 3-12.

Table A-5 Peak intensities and HA concentration of mature samples.

Sample	Int. (a.u.)	Conc.(mg/L)	Sample	Int. (a.u.)	Conc.(mg/L)
AT 0 (a)	17.04	129.75	**A 0 (a)**	15.52	119.76
AT 0 (b)	15.85	121.89	**A 0 (b)**	16.82	128.28
AT 0 (c)	16.77	127.97	**A 0 (c)**	15.45	119.27
AT 5 (a)	17.40	132.12	**A 5 (a)**	16.29	124.78
AT 5 (b)	15.65	120.62	**A 5(b)**	12.45	99.53
AT 5 (c)	13.05	103.49	**A 5 (c)**	16.91	128.91
AT 10 (a)	18.30	138.00	**A 10 (a)**	15.25	117.98
AT 10 (b)	16.21	124.27	**A 10 (b)**	15.70	120.92
AT 10 (c)	15.13	117.17	**A 10 (c)**	16.43	125.75
AT 20 (a)	20.52	152.58	**A 20 (a)**	16.83	128.36
AT 20 (b)	18.17	137.15	**A 20 (b)**	13.77	108.20
AT 20 (c)	18.43	138.87	**A 20 (c)**	14.28	111.56
AT 50 (a)	15.98	122.79	**A 50 (a)**	14.44	112.63
AT 50 (b)	16.35	125.17	**A 50 (b)**	15.35	118.63
AT 50 (c)	15.48	119.48	**A 50 (c)**	17.02	129.62

Table A-6. Average values of HA concentration for the different treatments.

Sample	Concentration (mg/L)		Sample	Concentration (mg/L)	
	Av	St Dev		Av	St Dev
AT 0	126.54	4.12	**A 0**	122.44	5.07
AT 5	118.74	14.41	**A 5**	126.84	2.92
AT 10	126.48	10.59	**A 10**	121.55	3.92
AT 20	142.87	8.46	**A 20**	116.04	10.80
AT 50	122.48	2.86	**A 50**	120.29	8.61

Table A-7. Concentration normalized to the FA standard carbon content values of concentration.

Sample	Concentration (mg/L)		Sample	Concentration (mg/L)	
	Av	St Dev		Av	St Dev
AT 0	91.22	2.97	**A 0**	88.27	3.65
AT 5	85.60	10.39	**A 5**	91.44	2.10
AT 10	91.18	7.63	**A 10**	87.62	2.83
AT 20	102.99	6.10	**A 20**	83.65	7.79
AT 50	88.29	2.06	**A 50**	86.72	6.21

A.1.6 Results for FA quantification of mature samples

The samples diluted to 1:25 prior to analysis. Intensity values of peak with Ex/Em 268/450 nm/nm and calculated FA concentrations are given in Table A-8. The FA concentrations were calculated by multiplying the concentrations obtained for the measured intensities with the dilution factor. The average values for the different treatments are given in Table A-9. The concentration normalized to the carbon content of the FA standard. The assumption that the carbon content of measured FA is 44% was made based on the measured concentration of carbon in HA, Normalized concentrations are given in Table A-10. Furthermore the concentrations converted to mg/g of compost. The results are given in Table 3-8 and Table 3-11.

Table A-8. Peak intensities and FA concentration of mature samples.

Sample	Int. (a.u.)	Conc.(mg/L)	Sample	Int. (a.u.)	Conc.(mg/L)
AT 0 (a)	77.34	109.91	**A 0 (a)**	74.01	104.77
AT 0 (b)	68.61	96.40	**A 0 (b)**	84.29	120.69
AT 0 (c)	79.63	113.47	**A 0 (c)**	74.00	104.74
AT 5 (a)	57.43	79.07	**A 5 (a)**	67.75	95.07
AT 5 (b)	60.30	83.52	**A 5(b)**	57.83	79.69
AT 5 (c)	56.71	77.96	**A 5 (c)**	72.68	102.71
AT 10 (a)	67.00	93.90	**A 10 (a)**	72.03	101.70
AT 10 (b)	66.74	93.49	**A 10 (b)**	65.44	91.48
AT 10 (c)	63.03	87.74	**A 10 (c)**	80.23	114.40
AT 20 (a)	87.26	125.29	**A 20 (a)**	55.64	76.30
AT 20 (b)	91.77	132.28	**A 20 (b)**	57.97	79.91
AT 20 (c)	92.72	133.75	**A 20 (c)**	64.55	90.10
AT 50 (a)	73.83	104.49	**A 50 (a)**	60.79	84.28
AT 50 (b)	77.98	110.91	**A 50 (b)**	65.60	91.73
AT 50 (c)	66.35	92.89	**A 50 (c)**	79.72	113.61

Table A-9. Average values of FA concentration for the different treatments.

Sample	Conc. (mg/L)		Sample	Conc. (mg/L)	
	Av	StDev		Av	StDev
AT 0	106.60	9.01	**A 0**	110.06	9.20
AT 5	80.18	2.94	**A 5**	92.49	11.72
AT 10	91.71	3.44	**A 10**	102.53	11.48
AT 20	130.44	4.52	**A 20**	82.10	7.16
AT 50	102.76	9.13	**A 50**	96.54	15.24

Table A-10. Concentration normalized to the FA standard carbon content values of concentration.

Sample	Conc. (mg/L)		Sample	Conc. (mg/L)	
	Av	St Dev		Av	St Dev
AT 0	92.97	7.86	**A 0**	95.99	8.02
AT 5	69.93	2.57	**A 5**	80.66	10.22
AT 10	79.99	3.00	**A 10**	89.42	10.02
AT 20	113.76	3.94	**A 20**	71.61	6.24
AT 50	89.62	7.97	**A 50**	84.2	13.29

APPENDIX B:GERMINATION BIOASSAYS

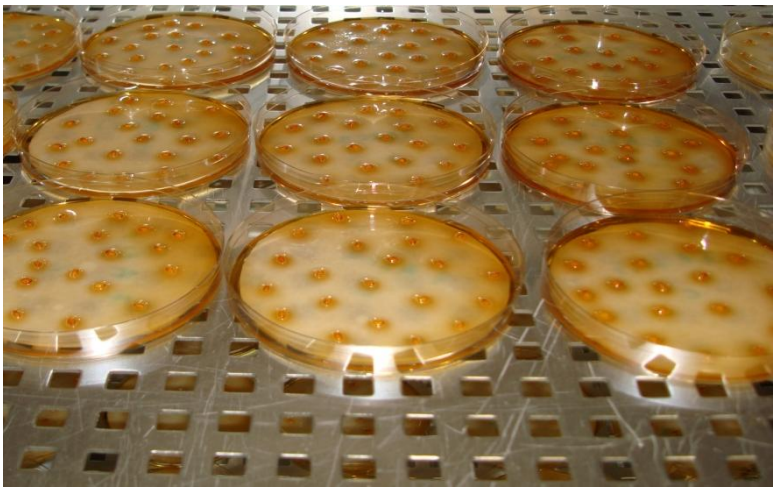

Fig B-1. Cress germination test at day 0.

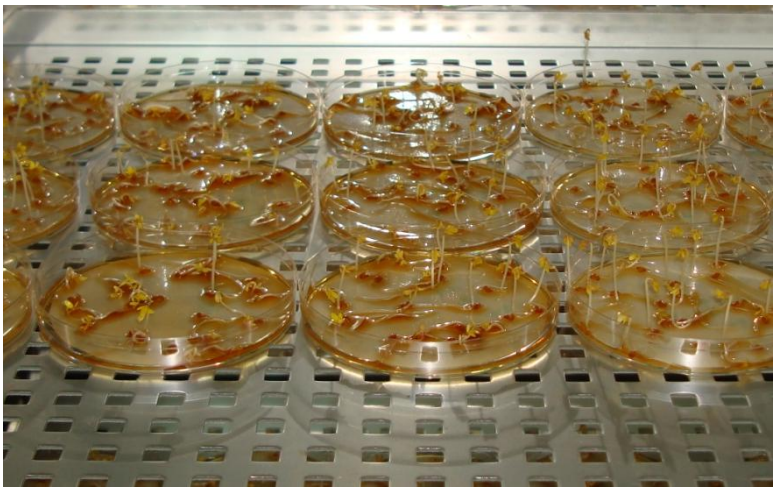

Fig B-2. Cress germination test at day 3.

Table B-1. Root elongation calculated for the experiments using NP-contaminated compost extracts.

Root Elongation							
	AT 0 (a)	**AT 0 (b)**	**AT 0 (c)**		**A 0 (a)**	**A 0 (b)**	**A 0 (c)**
1	56.94	66.55	60.80	1	54.10	43.75	40.90
2	70.00	51.32	55.00	2	65.37	40.79	44.10
3	64.67	56.70	69.63	3	55.37	45.85	41.30
Av	**63.87**	**58.19**	**61.81**	**Av**	**58.28**	**43.46**	**42.10**
StDev	6.57	7.72	7.37	StDev	6.17	2.54	1.74
	AT 5 (a)	**AT 5 (b)**	**AT 5 (c)**		**A 5 (a)**	**A 5 (b)**	**A 5 (c)**
1	60.75	71	73	1	64.30	60.85	43.90
2	73.25	65.95	72	2	57.00	62.80	50.94
3	68.79	63.59	68.65	3	69.50	63.68	49.20
Av	**67.60**	**66.85**	**71.22**	**Av**	**63.60**	**62.44**	**48.01**
StDev	6.33	3.79	2.28	StDev	6.28	1.45	3.67
	AT 10 (a)	**AT 10 (b)**	**AT 10 (c)**		**A 10 (a)**	**A 10 (b)**	**A 10 (c)**
1	66.84	68.61	57.63	1	55.15	60.00	74.65
2	61.58	59.12	56	2	58.35	57.85	75.10
3	62.89	63.95	55	3	54.45	55.10	70.90
Av	**63.77**	**63.89**	**56.21**	**Av**	**55.98**	**57.65**	**73.55**
StDev	2.74	4.75	1.33	StDev	2.08	2.46	2.31
	AT 20 (a)	**AT 20 (b)**	**AT 20 (c)**		**A 20 (a)**	**A 20 (b)**	**A 20 (c)**
1	62.35	61.95	56.5	1	68.15	41.70	39.70
2	54.15	63.4	60.6	2	64.45	52.35	39.32
3	n/a	n/a	n/a	3	60.55	43.70	37.79
Av	**58.25**	**62.68**	**58.55**	**Av**	**64.38**	**45.92**	**38.94**
StDev	5.80	1.03	2.90	StDev	3.80	5.66	1.01
	AT 50 (a)	**AT 50 (b)**	**AT 50 (c)**		**A 50 (a)**	**A 50 (b)**	**A 50 (c)**
1	62.45	58.80	58.50	1	54.37	56.00	39.95
2	57.85	51.50	50.16	2	54.00	48.45	38.45
3	63.00	54.32	56.10	3	61.68	57.40	37.84
Av	**61.10**	**54.87**	**54.92**	**Av**	**56.68**	**53.95**	**38.75**
StDev	2.83	3.68	4.29	StDev	4.33	4.81	1.08

Table B-2. Germination Index calculated for the experiments using NP-contaminated compost extracts.

Germination Index (%)			
AT 0 (a)	136.98	A 0 (a)	147.59
AT 0 (b)	131.49	A 0 (b)	111.97
AT 0 (c)	139.67	A 0 (c)	110.30
Av	**136.05**	**Av**	**123.29**
StDev	4.17	StDev	21.07
AT 5 (a)	161.48	A 5 (a)	166.62
AT 5 (b)	151.57	A 5 (b)	160.87
AT 5 (c)	172.99	A 5 (c)	121.60
Av	**162.01**	**Av**	**149.70**
StDev	10.72	StDev	24.50
AT 10 (a)	154.89	A 10 (a)	146.67
AT 10 (b)	142.22	A 10 (b)	151.03
AT 10 (c)	134.27	A 10 (c)	192.69
Av	**143.79**	**Av**	**163.46**
StDev	10.40	StDev	25.40
AT 20 (a)	140.46	A 20 (a)	165.86
AT 20 (b)	151.15	A 20 (b)	120.30
AT 20 (c)	141.19	A 20 (c)	100.30
Av	**144.27**	**Av**	**128.82**
StDev	5.97	StDev	33.60
AT 50 (a)	138.06	A 50 (a)	143.55
AT 50 (b)	123.99	A 50 (b)	141.34
AT 50 (c)	124.10	A 50 (c)	98.13
Av	**128.72**	**Av**	**127.67**
StDev	8.09	StDev	25.61

Table B-3. Root Elongation and Germination Index calculated for the experiments conducted using NPs solutions.

Root Elongation (mm)						
Ag-TiO2-NPs						
	5 mg/L	**10 mg/L**	**20 mg/L**	**30 mg/L**	**100 mg/L**	**Pure Water**
1	43.75	35.25	32.25	28.50	11.89	39.00
2	42.75	36.25	33.00	32.89	16.74	41.25
3	38.25	38.75	31.75	27.25	14.58	43.25
Av	**41.58**	**36.75**	**32.33**	**29.55**	**14.40**	**41.17**
StDev	**2.93**	**1.80**	**0.63**	**2.96**	**2.43**	**2.13**
AgNPs						
	5 mg/L	**10 mg/L**	**20 mg/L**	**30 mg/L**	**100 mg/L**	**Pure Water**
1	39.90	40.50	34.85	32.65	28.68	40.40
2	39.45	38.00	42.55	38.10	30.05	38.45
3		38.50	40.00	33.85	30.40	37.25
Av	**39.68**	**39.00**	**39.13**	**34.87**	**29.71**	**38.70**
StDev	**0.32**	**1.32**	**3.92**	**2.86**	**0.91**	**1.59**
Germination Index (%)						
Ag-TiO2-NPs						
	5 mg/L	**10 mg/L**	**20 mg/L**	**30 mg/L**	**100 mg/L**	**Pure Water**
1	106.27	85.62	78.33	69.23	28.89	100.00
2	103.84	88.05	80.16	79.90	40.65	100.00
3	92.91	94.12	77.12	66.19	35.41	100.00
Av	**101.00**	**89.26**	**78.54**	**71.77**	**34.99**	**100.00**
StDev	**7.12**	**4.38**	**1.53**	**7.20**	**5.89**	**0.00**
AgNPs						
	5 mg/L	**10 mg/L**	**20 mg/L**	**30 mg/L**	**100 mg/L**	**Pure Water**
1	103.10	104.65	90.05	84.37	71.64	100.00
2	101.94	98.19	109.95	98.45	75.05	100.00
3	n/a	99.48	103.36	87.47	75.92	100.00
Av	**102.52**	**100.78**	**101.12**	**90.09**	**74.20**	**100.00**
StDev	**0.82**	**3.42**	**10.14**	**7.40**	**2.27**	**0.00**

APPENDIX C: PARTICLE SIZE DISTRIBUTION

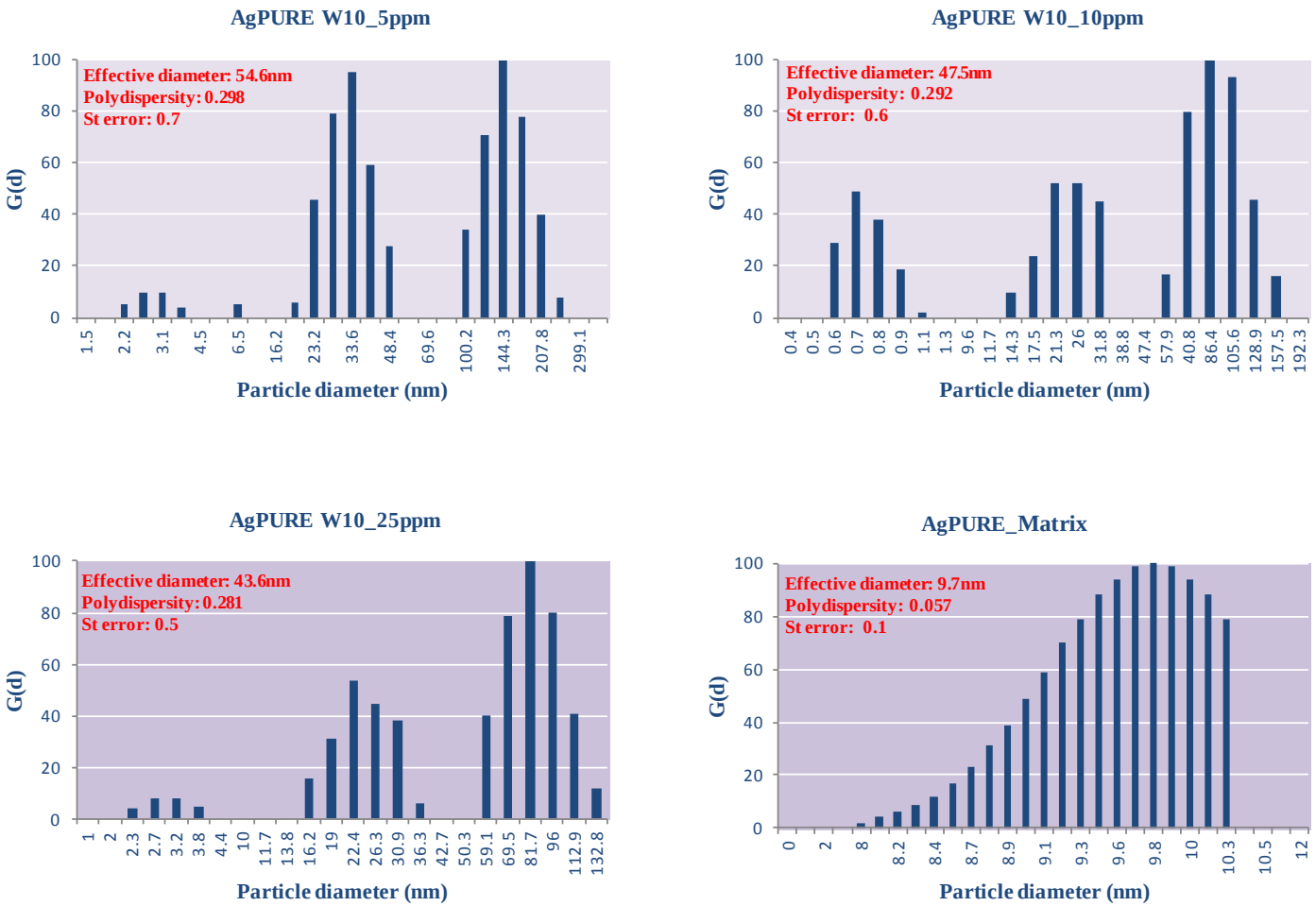

Fig C-1. Particle size distribution for AgNP solutions at concentrations of 5, 10 and 25 mg Ag / L and for the matrix.